5-1982

Seed Case Studies on Atriplex Gardneri (MOQ) Dietr.: Bracteole Cell Wall Composition and Enzymatic Degradation

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SEED CASE STUDIES ON ATRIPLEX GARDNERI (MOQ) DIETR.: BRACTEOLE CELL WALL COMPOSITION AND ENZYMATIC DEGRADATION

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science

UTAH STATE UNIVERSITY

Logan, Utah

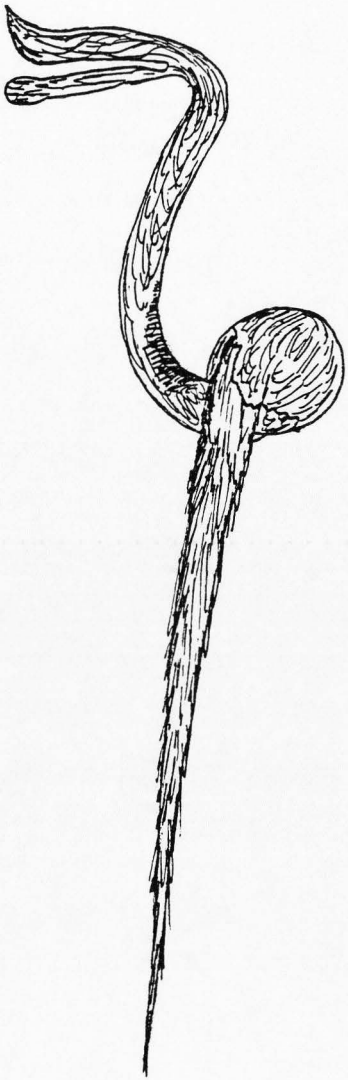
1982

DEDICATION

Now, as I live,
I fondly remember you.

With love, to

Herbert Ballantyne Woolley



ACKNOWLEDGEMENTS

Throughout my stay at Utah State University, my livelihood and research have been funded by several different organizations: the Philanthropic Educational Organization (P.E.O.) contributed an educational loan, the School of Graduate Studies awarded a graduate research fellowship, the National Science Foundation (DEB-79-04534) and the Northern Energy Resources Corporation (C19178128) granted research funds. For all of the support, I am much obliged. In particular, I would like to thank Bill Campbell for all of the effort that went into generating funds and for the very fine attribute of allowing his students free reign.

All of my committee members deserve mention and thanks. Anne Anderson, Bill Campbell, and Scott Bittner all served (willingly) as a source of information and encouragement. Special thanks go to Frank Burton and Scott Bittner whose collective wisdom and advice were crucial to the completion of the project.

Priscilla W. Burton

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ABSTRACT

Seed Case Studies on Atriplex gardneri (Moq) Dietr.:

Bracteole Cell Wall Composition and

Enzymatic Degradation

by

Priscilla Woolley Burton, Master of Science

Utah State University, 1982

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Department: Plant Science

Clarification of the bracteole restraint on germination and the development of an improved seed pre-treatment were the objectives of this study. Bracteole cell walls were isolated and hydrolyzed with 72% sulfuric acid followed by dilution and further hydrolysis. Individual monosaccharide yields were monitored with gas chromatography and colorimetry. Lignin was determined as the acid insoluble residue. Cell wall composition on a dry weight basis was 17.0% lignin, 32.75% glucose, 28.77% xylose, 7.21% arabinose, 1.11% galactose and 0.35% mannose. Linear xylans and glucans represent a large portion of the cell wall. The quantity of lignified tissue is extraordinarily large, resulting in a rigid, impermeable seed case.

Bracteole cell walls were delignified with acid chlorite. A comparison of enzyme catalyzed hydrolysis of the untreated cell walls with delignified cell walls followed. Three commercial enzyme

preparations were incubated with the samples separately and in combination treatments: cellulase (EC 3.2.1.4) from Trichoderma viride supplemented with B-D-glucosidase (EC 3.2.1.21); crude-hemicellulase from Aspergillus niger; polygalacturonase (EC 3.2.1.15) from Aspergillus niger. The production of reducing groups was monitored. Enzyme catalyzed hydrolysis of lignified cell walls was limited: cellulase followed by polygalacturonase degraded 14.1% of the lignified cell wall, all other treatments yielded less than 10% degradation. Dramatic improvements in the activity of all enzymes was noted after delignification. Cellulase activity increased to 35%, hemicellulase to 25%, and polygalacturonase to 36% hydrolysis of the sample.

Intact seeds were subjected to delignification with acid chlorite at 35 degrees. Of these seeds, half were treated with cellulase, supplemented as before. The germination percentages of delignified and delignified/enzyme treated seeds were compared with other common germination pre-treatments. Four hundred seeds of each treatment were sown in soil and allowed to germinate with 20 degree days and 15 degree nights. The thirty minute delignification left the seed viable, but still lignified: only 3% germinated as compared with 28.0% for physically scarified seeds, 6% for acid treated seeds, 5.5% for surface sterilized seeds, and 4.0% for cold water leached seeds. Physical scarification proved to be the easiest method of pre-treatment.

(86 pages)

INTRODUCTION

Atriplex species are utilized in reclamation efforts on semi-arid lands owing to their drought and salt tolerance and their palatability to livestock. Atriplex gardneri is a low growing shrub with a deep tap root system that stabilizes soil banks. The species is native to the western U.S. and thereby qualifies for use under the Surface Mining Control and Reclamation Act of 1977, P.L. 95-87. Unfortunately, propagation of the plant by seed is difficult and knowledge of germination has scarcely improved in the last 28 years.

Atriplex species are dioecious. The incomplete, pistillate flower consists of a naked pistil formed in the axis of two bracteoles. The bracteoles are photosynthetically active, modified leaves, that envelope the seed as the flower senesces, hardening into very tough, protective structures. The seed is not released from the bracteoles upon maturation, but must await natural or artificial removal of the bracteoles before germination.

Estimates of average percent germination of filled fruits varies according to species and treatment. The available data are sketchy; Table 1 provides a summary of the species tested and percent germination along with the treatment applied. Percent germination was tremendously increased in trials where the seeds were excised or debracted. This is the crux of the seed propagation problem. The bracteoles are responsible for a mechanical restraint to germination.

Table 1. Average percentage germination of filled seed according to species and treatment.

Species	Treatment[a]	Germination (%)	Source
A. canescens	9.44 to 17.2 °C	27	Springfield 1966
	dewinged seed, 12.3 to 1.6 °C	81	Crofts 1977
A. confertifolia	"	19	Crofts 1977
A. gardneri	"	87	Crofts 1977
A. lentiformis	10/25 °C alternation for 16/8 hours	65	Young et al 1980
A. nummelaria	excised seed, 20 °C and 0 to -2.0 atm (PEG 20,000)	95	Sharma 1976
A. patula	presoaked in water, 0/10 or 5/25 °C alternation for 16/8 hours	77	Young et al 1980
A. polycarpa	-0.42 atm (NaCl)	61	Chatterton and McKell 1969
	presoaked in tapwater and 20 °C	15	Cornelius and Hylton 1969
A. repanda	excised seed, pierced testa	93	Lailhacer-Kind and Laude 1975
A. semibaccata	presoaked in tapwater, 10/25 °C alternation for 16/8 hours	68	Young et al 1980
A. vesicaria	excised seed, 20 °C, 0 to -1.0 atm. (PEG 20,000)	90	Sharma 1976

[a]Unless specified, seeds were contained in the bracteoles. Dewinged refers to clipping a portion of the bracteoles, excised refers to complete removal of the bracteoles.

The description of cultured sycamore cell walls (Albersheim, 1976) serves to represent the primary cell wall model for all higher plants, even at present (McNeil et al. 1979). The model visualizes cellulose fibers coated with hemicellulosic polymers and interconnected by the covalent linkage of pectic polymers and glycoproteins. In the secondary wall, this structure is enlarged by several layers of cellulose fibers, the hemicellulosic content is increased, and an ingression of lignin occurs (Cote, 1977; Northcote, 1972). Lignification results in a very rigid structure resistant to impact and compression, and impermeable to oxygen or water.

Regularly repeating linkages, such as those in cellulose, can be degraded by specific enzymes. Lignin, a polymeric phenol, has no regularly repeating linkages, and is resistant to microbial decay (Freudenberg, 1966; Hartley, 1978). Yet, certain pathogens, e.g. the white rot fungi (Crawford and Crawford, 1980) are known to completely degrade the lignin in the bark of trees. Apparently, these fungi utilize oxidative and hydrolytic processes concurrently (Hall, 1980; Kirk and Chang, 1981; Crawford and Crawford, 1980).

Presently, hard-seededness is treated with physical or chemical scarification methods that have been shown to be ineffective or detrimental to the developing Atriplex embryo (Crofts, 1977; Lailhacer-Kind and Laude, 1975). Enzymes are more efficient agents of hydrolysis than acids (Ghose and Pathak, 1973). Therefore, cell-free culture filtrates of fungal enzymes might be used to perforate the lignin structure of the Atriplex bracteoles, improving contact of the embryo with environmental moisture and oxygen.

Elucidation of the bracteole cell wall composition will provide an understanding of the origin of mechanical germination inhibition and a basis for developing an enzymatic pre-germination treatment scheme. The quantity of lignin in the bracteole cell walls should indicate their susceptibility to enzymatic digestion. The objectives of this study are to 1) hydrolyze the bracteole cell wall polysaccharides into their constituent monomers for indentification 2) to estimate the lignin content 3) to biologically degrade the cell wall and 4) to assess the effectiveness of the biological method developed on intact seeds as a pre-germination treatment.

REVIEW OF LITERATURE

Atriplex gardneri (gardner saltbush) is a member of the family Chenopodiaceae.[1] Of the 102 known genera in the family, only eighteen occur in the western U.S. and several of these are considered salinity indicators: Salsola, Suaeda, and Salicornia (Lawrence, 1951). A well known family member is Beta vulgaris L, the common beet.

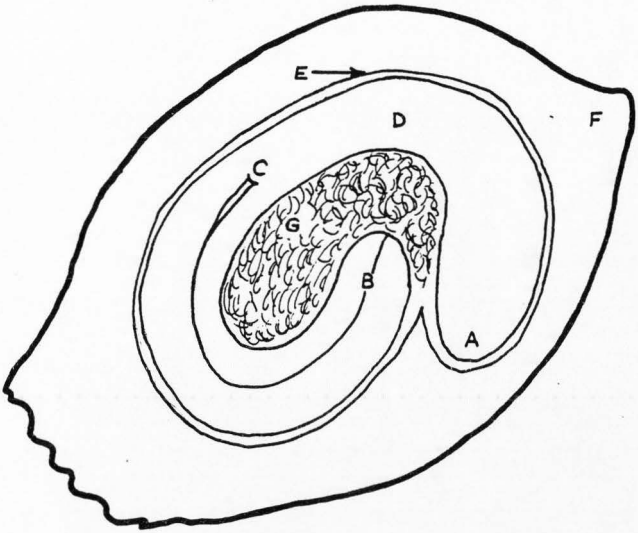
The family Chenopodiaceae is characterized as halophytic annuals and perennials. They are commonly found in arid or semi-arid regions receiving only 2.5 cm of rainfall annually (Beadle, 1952; Chatterton et al. 1971; Stark and Love, 1969). These plants can exist in dry, stony soils with extremely dry air and persistant winds as a result of their phreatophytic habits. Moisture requirements are fulfilled as diurnal temperature extremes create condensation under rock surfaces and as ground water vapor is distilled (Stark and Love, 1969). Measurements of water potential in the zone of root growth reveal tolerance of soil osmotic pressure to minus 25 bars (Chatterton et al. 1971) and of internal plant water potentials of minus 39 bars (Richardson and McKell, 1980). These halophytes not only tolerate

[1]The species is sometimes refered to as Atriplex gardoni. Apparently, Moquin used "gardneri by mistake" (Dorn, 1977). However the species is often listed as A. gardneri (Holmgren and Reveal, 1966) and contains members originally identified as A. nuttali, A. falcata, A. fruticulosa, A. eremicola, A. buxifolia, A. pabularis, A. tridentata, and A. oblanceolata (Dorn, 1977).

high salinity levels, but require saline conditions for optimum growth. Greenway (1968) found the optimum growth of A. hymenelytra to be obtained with solute levels of 100 meq/L where plants were cultured in liquid solution of sodium chloride and nutrients.

Atriplex species are dioecious, having imperfect, incomplete flowers that are minute and greenish in color (Pool, 1929). The staminate flowers are clustered on a common peduncle in axillary glomerules on spikes. These staminate flowers are bractless. The five stamens are surrounded by a five-parted calyx. A corolla does not form. The pistillate flower has no calyx or corolla. Instead, a naked pistil, with two styles, two or three fused carpels, and one ovule, is produced in the axis of two bracteoles. The ovule is campylotropous in form (Davis, 1966), which produces an embryo curled around the perisperm (Figure 1).

The fruit of the family Chenopodiaceae is described as an indehiscent nutlet that contains only one seed. The testa develops from the integuments of the ovule. In the Chenopodiaceae, there are two integuments, each two cells in thickness. The outer integument cells become filled with brown resinous material. A cuticular layer develops on both the outer and innermost surfaces of the integuments; a total of three layers are produced. The cuticle covering ends in the funicular region, where vascular tissue occurs (Davis, 1966; Esau, 1953). The bracteoles envelope the seed as the flower senesces, hardening into very tough, protective structures. The vascular and parenchyma tissue in the funicular region dehydrates to form a plug of loosely connected cells (Perry and Harrison, 1974).



A— RADICLE
B— COTYLEDONS
C— SHOOT APEX
D— HYPOCOTYL
E— TESTA
F— BRACTEOLES
G— PERISPERM

Figure 1. The curled embryo of Atriplex.

The pore, located at the base of the seed, is the only entrance for water and oxygen in the otherwise sclerified bracteoles. The seed is not released from the bracteoles upon maturation, but must await natural or artificial removal of the bracteoles before germination.

The genus Atriplex has an extremely low percentage of fruit fill. Springfield (1967) found that of 16,000 A. canescens seeds, 53.6% was the average fill rate. Many fruits contain no evidence of fertilization, only the shrunken remains of the pistil. This phenomenon may be due to any number of adverse environmental conditions, particularly high winds preventing contact of pollen and stigma and drought hindering the development of the embryo. The bracteoles envelope the gynoecium at the end of the flowering season despite the absence of a developing embryo, which may explain the abnormally high percentage of empty seed cases.

Earlier research has centered around the chemical inhibition of germination caused by the high concentrations of sodium chloride (Beadle, 1952) and calcium oxalate crystals in the cell vacuoles (Vest, 1952). The sodium chloride concentration was determined to be 12 - 13% of the total dry weight of the seed of Atriplex dimorphostegia (Koller, 1957). Osmotic inhibition can be easily relieved through leaching and pre-drying the seeds before sowing (Beadle, 1952; Vest and Cottam, 1953; Nord and Whitacre, 1957). In the laboratory, 24 hours of leaching in running tap water should suffice (Vest, 1952). Yet, Vest (1952) found inhibition of germination even after 24 hours of leaching A. canescens seed. The leached seed was assumed free

from all soluble inhibitors, since excised seeds germinated in the presence of the leached seeds. (Previous trials had demonstrated that excised seeds would not germinate in the presence of untreated, intact seeds or bracteoles.) Vest theorized that the seed case was impregnated with calcium oxalate that mechanically restrained the swelling embryo.

The calcium oxalate theory has never been pursued. The highly sclerified cell walls of the bracteoles are the more probable cause of mechanical restriction. Attempts to increase germination through degradation of the cell walls include hammermilling (Crofts, 1977; Nord and Whitacre, 1957) and scarification with sulfuric acid and sodium hypochlorite (Lailhacer-Kind and Laude, 1975). These treatments were found to increase germination, but were not completely effective or acceptable. Hammermilling resulted in the loss of approximately 27% of viable A. canescens seed and 50% of viable A. confertifolia seed (Crofts, 1977). Crofts (1977) also reported the tendency toward abnormal germination was increased. The approximate average rate of radicle emergence only was 20% and epicotyl emergence only was 28%.

Angiosperm cell wall structure and components

Responsibility for the strength of the seed case lies with the composition and structure of the cell walls. Primary cell walls of distantly related plants have many common features (McNeil et al. 1979). The primary cell wall is organized by a framework of cellulose fibrils which are completely encased by compatible, linear hemicelluloses,

hydrogen-bonded to their surfaces, possibilities incluce: B-1,4-glucans, xylans, mannans or xyloglucans (McNeil et al. 1979; Keegstra et al. 1973; Preston, 1979). The coated fibrils are cross-linked by a matrix of hemicellulosic and pectic polymers progressively less ordered: twisted ribbon-like chains, helical chains and completely random chains (Preston, 1979; Talmadge et al. 1973). An excellent discussion of the structure and interaction of primary cell wall polysaccharides was written by Preston (1979).

As a cell differentiates, the synthesis, packing density and orientation of microfibrils and matrix polysaccharides is under membrane control (Northcote, 1972). Thus, secondarily thickened cell walls vary between cell types. Unlike primary cell walls, secondary walls are formed once elongation of the cell has ceased (Wardrop, 1971). Secondary cell walls have densely packed cellulose fibrils laid down in two or three distinct lamellae, visible with the electron microscope. Adjacent layers are each oriented differently, but laminated together to create a unit which has strength in more than one direction. Lamellation may be the result of changes in cellulose content between layers, changes in cellulose fiber orientation, and/or changes in type of non-cellulosic substances (Preston, 1974). The hemicellulose synthesized during secondary wall formation is predominantly B-1,4-mannan in gymnosperms and B-1,4-xylan in angiosperms (Northcote, 1972).

At approximately the same time of secondary wall formation, lignification begins to occur in the cell corners, just within the primary cell wall (Wardrop, 1971). Lignification continues through

the middle lamella and the secondary wall. It is the final process in wall formation. As lignin penetrates the wall, it replaces space otherwise occupied by structural water (Northcote, 1972) thoroughly encrusting the matrix and fibrils of the wall. As bonds between water and matrix polysaccharides are broken, there is an increased association between the matrix and fibers due to hydrogen-bonding, with a resulting increase in strength (Northcote, 1972).

Lignin is derived from enzymatic dehydrogenation and polymerization of coumaryl, coniferyl, and sinapyl alcohols (Gross, 1980). The phenolic precursors of lignin are probably synthesized in differentiating cells and translocated to the region of secondary cell wall thickening (Northcote, 1972). The polymerization occurs within the matrix of the wall via a free-radical mechanism and covalent lignin-carbohydrate bonds are formed. Consequently, the molecule is large, irregular, and inconsistantly bonded.

Hemicellulosic B-glucans are of three possible linkages. A polymer of mixed B-1,3 and B-1,4-linkages is known to exist in monocots and has been isolated from leaf, stem and endosperm tissue (McNeil et al. 1979). But, according to McNeil et al. (1979), there is only one report of such a compound in dicots. Buchala and Franz (1974) isolated a mixed 1,3 and 1,4 linked compound from three-day old cell walls of Phaseolus aureus (mung bean) hypocotyls. The compound could not be isolated from more mature tissue, however. Buchala and Franz (1974) suggest that as the cell wall matures, the mixed linkage polymer may be degraded by the action of glucanases or diluted by the formation of other plant hemicelluloses at maturity. The 1,3 and 1,4 linked glucan was extracted with hot water, indicating

non-covalent and weak hydrogen-bonding to the cellulose fibrils. According to McNeil et al.(1979), this mixed linkage glucan does not fall into the category of hemicellulosic compounds, which are defined as having strong hydrogen bonds to cellulose.

Callose (B-1,3-linked glucan) is found in granular deposits that form the pores of phloem sieve cells (Salisbury and Ross, 1978). Stimulation for B-1,3-glucan synthesis may be induced by mechanical damage or as a defense reaction to infection. Sheaths of B-1,3-glucan encapsulate invasive hyphae to prevent further penetration and plugs of the polymer form in the pores of plasmadesmata to arrest viral infection (Pegg, 1976). B-1,3-glucan is even formed within the stigma to arrest the passage of incompatible pollen grains (Pegg, 1976). B-1,3-glucan is often found associated with trace amounts of uronic acids (Aspinall, 1980). The linkage in callose forces the glucan to assume a helical conformation that physically entraps other matrix polymers (Preston, 1979).

Hemicellulosic B-1,4-linked glucan is a linear molecule with a lower degree of polymerization than the cellulosic B-1,4-glucan chain. The hemicellulosic B-1,4-linkage is chemically indistinguishable from that in cellulose (Preston, 1979). The polymer may actually be a cellulose precursor or degradation product.

Xyloglucans have been isolated from a number of sources: in Tamarindus indica seeds (Kooiman, 1961); Brassica campetris (rape seed) (Aspinall et al. 1977); Acer pseudoplatanus (sycamore) cells (Bauer et al. 1973); and in the exudate of cultured phaseolus vulgaris (red kidney bean, Wilder and Albersheim, 1973). Regardless of origin, xyloglucans commonly have a B-1,4-linked glucan backbone, with xylose

residues attached in an α-1,6-linkage to the glucan chain. At least one-half of the glucan chain is substituted in this manner (a feature that separates polymers of xyloglucan from B-1,4-glucan). Ratios of xylose:glucose range from 0.75 in Tamarindus, 0.71 in Brassica, to 1.16 in Acer (calculated on a mole percentage basis, McNeil et al. 1979). Comparison of these fractions is difficult, because methods of isolation varied. Xyloglucans may contain fucosyl, galactosyl or arabinosyl residues (Aspinall, 1980). Only 50% of isolated xyloglucans have been substituted by fucosyl residues, however. The speculation is that during secondary wall formation, the fucosyl residues are removed (McNeil et al. 1979).

The most abundant non-cellulosic polysaccharide in the secondary cell walls of plants is the B-1,4-linked xylan (Aspinall, 1980). Xylan is one of few polysaccharides that is incorporated into wall structure during both primary and secondary wall formation (Northcote, 1972). The B-1,4-linkage of xylan is not as strong as the B-1,4-glucan because the xylan chain twists slightly. So that, after three xylose residues, a 180° turn has been made in the xylan backbone (Van Soest, 1982). Aggregations of chains are stabilized by water molecules, with the inner hydrophilic portions accomodating the uronic acid and arabinose sidechains. When grouped in this manner, xylan chains could be oriented in the direction of the cellulose fibers and hydrogen-bonded to the cellulose chain (Northcote, 1972). The degree of polymerization of xylan chains is approximately 150 to 200 (Northcote, 1972).

Xylan chains are usually substituted with short sidechains, generally of three types: 1) glucuronic acid, 2) single L-arabinose residues and 3) lengthened chains of L-arabinose with a variety of other substituents (Aspinall, 1980). Monocot xylans have L-arabinose units substituted α-1,3 in addition to 4-O-methyl-D-glucuronic acid substituted α-1,2 (Northcote, 1972). Aspinall (1980) reports that dicot xylans are infrequently substituted with arabinose units, singly or otherwise. The most common substituent in dicot xylans is the 4-O-methyl-D-glucuronic acid, approximately 10% of the polymer is substituted (Aspinall, 1980).

The pectin category of cell wall polysaccharides incorporates neutral and acidic polymers located in the middle lamella and primary cell walls, as well as the intercellular regions. Pectins usually comprise a major fraction of dicot cell walls (Van Soest, 1982). The various polysaccharides falling under the heading of pectin include: rhamnogalacturonans, arabinogalactans, arabinans, galactans, and glycoproteins (Aspinall, 1980; Clarke et al. 1979). The term pectin generally refers to chains of α-1,4-linked galacturonic acid interspersed with rhamnose. The rhamnose/galacturonic acid bond lends a characteristic twist to the polygalacturonic acid chain. The chains may be esterified with methoxyl groups. Polygalacturonic acids relatively free from esterification (less than 0.8% methoxyl content, Doesburg, 1973) are termed pectic acids or pectate (Versteeg, 1979). Those with more than a trace of methoxyl groups esterified are termed pectin or pectinic acids (Doesburg, 1973; Versteeg, 1979). Polygalacturonic acids may be substituted with neutral sugars, also by an ester linkage. The structural diversity of

these compounds results from a variety of linkages and combinations of substituents. Their common properties include a high molecular weight and a flexible, deformable backbone with regions of regular carbohydrate sequence that provide interaction with other molecules through hydrogen-bonding (Clarke et al. 1979). Their adhesive nature and their water-holding capacity are the basis for speculation on a wide variety of functions (Clarke et al. 1979).

The most abundant polysaccharide of the cell wall is cellulose. The stability of cellulose and its resistance to solubilization are a direct consequence of the B-1,4-glucan linkage (Van Soest, 1982) which allows adjacent glucose molecules to be trans to one another, permitting all the molecules in a chain to be in the same plane. Intermolecular hydrogen bonding between adjacent glucose molecules within a chain (from the hydroxyl of C-2 to the glycosidic oxygen of the adjacent molecule) stabilizes the long polymers of glucose (as many as 10,000 units, Preston, 1974). Glucan chains are aggregated by interchain bonding (hydroxyls on C-6) to form fibers approximately 60 chains and 4.5 X 8.5 nm in diameter (McNeil et al. 1979). Chang (1971) describes the organization of the protofibrils within a fiber as a platellite, wherein a cellulose chain folds back upon itself. Where the folds occur, two or three monomer units rotate around the C-O bond deflecting approximately 60° from the linear chain. The platellites are held together by hydrogen bonds and van der Waals forces. The loop configuration exposes the acetyl oxygen atoms of the molecules involved and may explain the existance of weak links in

the cellulose crystalline structure. The folding chain theory explains the mercerization of cellulose fibrils in alkali and the occurrence of a minimal degree of polymerization (LODP) below which no further degradation occurs with either acid or enzyme hydrolysis (Chang, 1971; Bisaria and Ghose, 1981). A major consequence of the folding chain theory is the anti-parallel layering of the cellulose fibrils. X-ray diffraction studies, however, reveal that the organization within a cellulose fiber is crystalline due to the parallel orientation of individual glucan chains (McNeil et al. 1979).

Lignin-carbohydrate complexes have been known to exist as intermediates in the isolates from Milled Wood Lignin (MWL). The composition of the carbohydrate fraction was similar to the total hemicellulose of extracted wood (Lai and Sarkanen, 1971). Azuma et al. (1981) extracted a lignin-carbohydrate complex with dioxane from MWL: 38% neutral sugars (L-arabinose, D-xylose, D-mannose, D-galactose, D-glucose), 6.2% uronic acids, and 51.5% lignin. The lignin could not be separated from the carbohydrate portion with gel filtration, electrophoresis, ultracentrifugation or hydrophobic interaction chromatography. Delignification with aqueous potassium borohydride yielded a lignin-carbohydrate complex in the filtrate from Jute fiber (Corchorus capsularis). The carbohydrate complex was glucuronoxylan, bound to lignin through a uronic acid ester bond (Das et al. 1981). Lai and Sarkanen (1971) postulated that there were three types of lignin-carbohydrate linkages: α-ether linkages (acid labile) to the hydroxyl groups of monosaccharide units; ester

linkages (hydrolyzed by mild alkaline treatments) to the carboxyl of glucuronic acid residues; and carbon-to-carbon or carbon-to-oxygen bonds resulting from radical coupling (resistant to hydrolysis).

Hydrolysis of the cell wall

Susceptibility to hydrolysis is dependent on the physical structure of the cell wall. Acid hydrolysis of polysaccharides is fast, due to the ability of the hydronium ion to permeate the cell wall capillaries. However, acid hydrolysis proceeds by non-specific degradation mechanisms (Philipp et al. 1979), cleaving acetal and hemi-acetal linkages of any type. Specific linkages can be degraded most efficiently through enzymatic action, requiring lower quantities of reagents for a given amount of product (Ghose and Pathak, 1973). The glycoproteins of hydrolase enzymes are large molecules when compared with hydronium ions, a molecular weight of 61,000 for the largest component of cellulase isolated from Trichoderma viride (Cowling and Brown, 1969). Consequently, diffusion and adsorbtion to the surface of their substrates is the limiting factor in enzymolysis (Cowling and Brown, 1969). Of paramount importance are the degree of crystallinity of cellulose and the degree of lignification of the cell wall. The crystallinity of cellulose is affected primarily by the degree of hydration of the glucose units within the fibril (Cowling and Brown, 1969; Philipp et al. 1979). Adsorbtion of moisture will increase swelling and maximize the size of fibril capillaries, providing a medium for diffusion and access to inner units of the fibril. A tremendous increase in the exposed surface

area results from hydration (Cowling and Brown, 1969). Hydration may also effectively deform the lattice structure, relieving conformation and steric rigidity and improving the likelihood for enzyme-substrate formation (Cowling and Brown, 1969; Atalla, 1979).

Limitations to both acid and enzyme hydrolysis result from shielding of polymers when tissues are lignified (Chesson, 1981; Cowling and Brown, 1969; Ford, 1978). Shielding results from encrustation and the chemical effects of lignin-carbohydrate covalent linkages (Chesson, 1981). Xylan polymers esterified with phenolic and uronic acid units, limit the swelling of polymers through cross-linkages and physically protect adjacent units from degradation with their large molecular dimensions (Tarkow and Feist, 1969; Das et al. 1981; Cowling and Brown, 1969).

Improvements in the degree of hydrolysis can be achieved through prior treatment of the plant sample. Physical treatments such as ball milling result in an increase in the available surface area and a decrease in crystallinity of woody samples (Dewey and Mandels, 1980). As a result of ball milling, a dramatic increase in enzyme activity on Red Oak wood samples was observed: from 6% to 93% hydrolysis of cellulose after 240 minutes of ball milling (Millett et al. 1979). Similar increases were noted from Douglas Fir and cotton linters pulp.

Alkali treatment has also been used successfully on herbage samples with a lower degree of lignification (Tarkow and Feist, 1969). Alkali treatment cleaves uronic acid, acetic and phenolic acid ester linkages, and partially solubilizes the hemicellulose present (Chesson 1981). Alkali does not cleave ether bonds, however. Alkali treatment at low temperatures results in a mercerized cellulose product. The fibril length is shortened and the fibrils are more densely packed. According to the folding chain theory (Chang, 1971) ions penetrate the platellite structure rupturing hydrogen bonds as glycosidic linkages are twisted. The folds of the platellite are increased in number with increasing ion concentration. After treatment with 1 M NaOH, Hartley and Dhanoa (1981) recorded twice as much degradation of Italian ryegrass with cellulase.

Delignification with sodium chlorite may be prefered (Brice and Morrison, 1982; Ford, 1978), because of its limited effects on the polysaccharides of the cell wall. Acidic chlorination has been used in the wood pulping process for many years. Elemental chlorine, the predominant species in an acidic environment, initiates the solubilization of lignin through substitution reactions at the aromatic ring of the phenylpropane units of lignin. A second substitution of chlorine to the aromatic ring cleaves hydroxyl or alkyl ether groups in the position alpha to the aromatic ring (O'Neil, 1962). The exact mechanism is not thoroughly understood, and may even involve cleavage of the phenolic ring itself (Collings et al. 1978). Ester bonds are broken, and partial degradation of lignin-carbohydrate complexes occurs (Brice and Morrison, 1982).

The extensive degradation of lignin is accomplished by the white-rot fungi (select members of the Basidiomycetes and Ascomycetes) only after several weeks of incubation (Crawford and Crawford, 1980). The pattern of decay follows this general scheme: 1) demethylation of aromatic units forming catechol (o-diphenolic moieties) 2) enzymatic ring cleavage in the ortho position and/or 3) non-enzymatic oxidation of diphenols. The fragments produced possess greater numbers of carboxyl and carbonyl groups and have a lower aromatic content than the starting material (Hall, 1980; Kirk and Chang 1981; Crawford and Crawford, 1980). Three enzymes have been shown to catalyze the oxidation of bonds within the lignin macromolecule, partially degrading the structure: laccase (1.10.3.2), peroxidase (1.11.1.7), and alcohol oxidase (1.1.3.13) (Hall, 1980; Crawford and Crawford, 1980). Although, the extent of enzyme participation in the delignification process is questioned by Hall (1980), who convincingly argues that the mechanism of decay is via enzyme initiated, highly reactive, diffusable entities: superoxide radicals, hydroxyl radicals, hydrogen peroxide and singlet oxygen. It is interesting to note that the above mentioned enzymes utilize singlet oxygen as an acceptor (laccase and alcohol oxidase) and hydrogen peroxide as an acceptor (peroxidase).

The hydrolysis of cellulose by the cellulase complex of Trichoderma origin is unparalleled by any other fungal enzyme. The complex can be fragmented into three distinct components: endo-1,4-B-glucanase (C_x) 3.2.1.4, exo-cellobiohydrolase (C_1 or CBH) 3.2.1.91, and B-glucosidase 3.2.1.21 (Li et al. 1965; Wood and McCrae, 1979).

The C_x and C_1 components can be further fractionated into at least four separate proteins (Gritzali and Brown, 1979). The theory behind the mode of attack on crystalline cellulose has been under considerable review (Wood and McCrae, 1979; Dewey and Mandels, 1980). The concensus of thought currently is that C_x and C_1 are both required for hydrolysis of crystalline cellulose, with the presence of B-glucosidase increasing activity through the removal of inhibitory end-products. C_x and C_1 may form a loose complex on the cellulose microfibril, C_1 initiates the attack on cellulose acting in a random fashion, dramatically reducing the degree of polymerization of the chain. C_1 must be present to immediately hydrolyze the non-reducing chain ends (generated by the action of C_x) into cellobiose units. Swiftness of C_1 is required, because the glucose molecules, held rigidly in place by intermolecular hydrogen-bonds, may reform the acetyle bond broken by C_x (Wood and McCrae, 1979).

The cellulase complexes of Trichoderma viride and Trichoderma reesei have very high concentrations of C_1 components (Gritzali and Brown, 1979; Wood and McCrae, 1979), but poor B-glucosidase activity (Humphrey, 1979). The C_x and C_1 union of cellulase is inhibited by the end-products of its activity, cellobiose. Therefore, addition of B-glucosidase to a culture filtrate will strongly enhance conversion of cellulose to glucose. B-glucosidase is in turn inhibited by the accumulation of its end-product, glucose. With industrial use of the enzyme, maximal hydrolysis is obtained by constant removal of decomposition products (Ghose and Pathak, 1973). Indications are that the

Trichoderma cellulase will catalyze the hydrolysis of B-1,4-glucan, B-1,3 and B-1,4 mixed linkage glucan polymers (Barras et al. 1969) and B-1,4-xylan linkages (Sinner et al. 1979). B-glucosidase of Trichoderma will catalyze the hydrolysis of B-1,2, B-1,3, B-1,6 or B-1,4 glucan bonds with the retention of configuration in the products (Dewey and Mandels, 1980).

Hemicellulase enzymes are as numerous as the variety of structures and linkages within the grouping of polysaccharides. Basically, there exist two forms of enzymes (Bisaria and Ghose, 1981) for each linkage, much like the cellulose decomposing enzyme complex of Trichoderma. An endo-type catalyzes random cleavage of the linkage, rapidly depolymerizing the chain length. An exo-type acts on the non-reducing ends, rapidly increasing the number of reducing sugars in solution. Both types of L-arabinases, D-mannases, D-galactanases and D-xylanases are thought to exist in fungal cultures (Bisaria and Ghose, 1981).

Research has centered on the use of D-xylanases, since xylan polymers are so ubiquitous. On complex substrates, xylananase acts synergistically with B-glucanases to enhance cellulase activity (Bisaria and Ghose, 1981; Sinner et al. 1979). Even electrophoretically pure exo-cellobiohydrolase contains some endo-xylanase activity (Sinner et al. 1979). Endo-1,4-B-D-xylanase (3.2.1.8) has been purified from crude hemicellulase preparations of Trichoderma reesei, T. roseum and Aspergillus niger (Bisaria and Ghose, 1981; Frederick et al. 1981; Sinner et al. 1979).

Bisaria and Ghose (1981) report that endo-xylanases may catalyze the hydrolysis of glucuronoxylans and B-1,4-xylans and some, but not all, may release arabinose from arabinoxylans and arabinoglucurono-xylans. Frederick et al. (1981) isolated an endo-xylanase that could not hydrolyze insoluble xylan; although, xylobiose and xylose were produced after incubation with a soluble, branched xylan polymer. No arabinose or free uronic acid was produced, indicating that the bonds close to the branch points were protected from hydrolysis. Sinner et al. (1979) incubated xylanase with delignified Sprucewood assuming the substrate to be arabino-4-O-methylglucuronoxylan. The products formed were xylobiose (predominantly) xylose, arabinose and oligosaccharides containing uronic acid residues. Possibly, the greatest inhibitor of activity is the presence of 4-O-methylglucuronic acid esters (Tarkow and Feist, 1969; Das et al. 1981).

The nomenclature of pectolytic enzymes is somewhat confusing. They are classified according to the degree of esterification of their preferred substrate, their mechanism of cleavage (transeliminative or hydrolytic), and their randomness of action (endo or exo). Table 2 lists the enzymes that have been isolated and identified as acting on polygalacturonide linkages. The specificity for pectin or pectic acid is not absolute -- neither is the distinction between the two substrates. Commercial preparations of enzymes are seldom pure (Rombouts and Pilnik, 1973; Reed, 1975; Karr and Albersheim, 1970).

Rombouts and Pilnik (1973) report the purification of endopoly-galacturonase, endopolymethylgalacturonase, pectin lyase, and a pectin-esterase from a commercial pectinase preparation of Aspergillus niger.

Reed (1975) reports that commercial preparations usually contain pectinesterase. Karr and Albersheim (1970) reported that the purification of a commercial pectinase preparation involved the separation of pectin hydrolase, pectin lyase, pectin esterase, polygalacturonic acid hydrolase, and pectic acid lyase in addition to several other polysaccharide degrading enzymes. After fractionation of this same preparation, Karr and Albersheim (1970) reported that prior treatment with an unidentified polygalacturonic acid degrading enzyme improved the succeptibility of Phaseolus vulgaris (red kidney bean) cell wall polymers to hydrolysis by other polysaccharide degrading enzymes. The conclusion that the enzyme cleaved cross-linkages between uronide constituents and polysaccharide polymers was substantiated by the fact that prior hydrolysis by acid or base substituted for the action of the unidentified enzyme.

Table 2. Polygalacturonide degrading enzymes as described by the Nomenclature Committee of the International Union of Biochemistry on the Nomenclature and Classification of Enzymes (1979).

Preferred Substrate	Name(s)[a]	E.C. Number	Reaction Catalyzed
Pectic Acid	POLYGALACTURONASE pectin depolymerase pectinase	3.2.1.15	random hydrolysis (endo) of 1,4-α-D-galacturonic acid linkages with the production of oligosaccharides
	EXOPOLYGALACTURONASE poly(galacturonate) hydrolase	3.2.1.67	end-wise hydrolysis (exo) of 1,4-α-D-galacturonic acid linkages with the production of D-galacturonate
	PECTATE LYASE pectate transeliminase	4.2.2.2	endo, transeliminative cleavage of Δ-4,5-D-galacturonic acid producing oligosaccharides
	EXOPOLYGALACTURONASE LYASE	4.2.2.9	exo, transeliminative cleavage of Δ-4,5-D-galacturonic acid, producing galacturonic acid
	EXO-POLY-α-D-GALACTURONOSIDASE	3.2.1.82	exo, hydrolysis, producing digalacturonate
Pectin	PECTIN LYASE	4.2.2.10	transeliminative cleavage of 6-methyl-Δ-4,5-D-galacturonic acid
	PECTINESTERASE pectin demethoxylase pectin methoxylase pectin methylesterase	3.1.1.11	demethylation of pectin producing pectic acid

[a]The name recommended by the Nomenclature Committee is written all in captial letters. The other names listed are commonly found in the literature.

MATERIALS AND METHODS

Atriplex gardneri seeds were collected from the Kemmerer Mine site, Kemmerer, Wyoming in July and August of 1980. The seeds were air-dried, and cleaned prior to storage at 4 C. The seeds were stored for one year before analysis.

A razor scalpel and forceps were used to separate the true seed -- embryo and testa -- from the seed case or bracteoles. The embryos were set aside for viability tests, and the bracteoles were collected for further testing. Seven grams of bracteoles were freeze-dried and ground in a cyclone mill equipped with a 40 mesh screen.

The percent dry matter was determined on three, 500 mg samples of ground bracteoles, that were weighed into three pre-weighed crucibles. The samples were dried in a vacuum oven at 60 C and 20 psi for 12 hours.

Approximately 2 grams of ground bracteoles were refluxed for one hour with 200 ml of 80% ethanol, while being stirred continuously with a magnetic stirring bar. The samples were filtered through a pre-weighed, 60 ml, sintered glass funnel (coarse porosity) with the aid of a water aspirator. The residue was washed with boiling 80% ethanol until the filtrate was colorless. The alcohol insoluble residue (AIR) was dried in a vacuum oven at 60 C and 20 psi for 12 hours.

After determination of percent AIR, the residue was returned to a round-bottom flask for refluxing in 200 ml of chloroform/methanol (1:1, v:v). After one hour, the sample was filtered (as described

above) and rinsed with boiling chloroform/methanol until the filtrate was clear. The sample was dried in a vacuum oven (as above) and the percentage of chloroform/methanol insoluble residue (CMIR) was noted (Woolard et al. 1977).

Cell wall hydrolysis

With the objective of determining the major components of the cell wall polysaccharides in mind, CMIR samples were hydrolyzed by the two-step H_2SO_4 acid hydrolysis procedure of Bittner et al. (1980). Three, 20 - 25 mg samples of the CMIR were weighed into 16 X 125 mm screw-capped test tubes. One ml of 72% H_2SO_4 was added (first step) to each tube and the contents were mixed, to ensure adequate contact between sample and acid, with the aid of a glass rod and vortex mixer. (The glass rod remained in the tube after mixing.) After 30 min of hydrolysis at room temperature with constant stirring, the test-tube solutions were brought to 2 N by the addition of 10 ml of distilled water (second step). Air was removed from the tubes with a stream of nitrogen (2 psi). The tubes were capped immediately and placed in a boiling water bath (95 C) for three hours. From one of the tubes, two 200 ul aliquots were drawn at 15 min, 30 min, 45 min, 60 min, 120 min, 180 min, and 240 min in preparation for a hydrolysis curve. After three hours, six, 200 ul aliquots were drawn from each of the remaining two tubes, after the tubes had been plunged into a cold water bath to end hydrolysis and centrifuged to precipitate the cell wall residue. The aliquots were transferred to 13 X 100 mm screw-capped tubes with a glass micropipet and analyzed for neutral and acidic sugars.

Three of the 200 ul aliquots containing liberated monosaccharides were neutralized with ammonia and reduced with sodium borohydride to form the corresponding alditols. The solution of alditols was acetylated with acetic anhydride and N-methylimidazole following the method of Bittner et al. (1980), with the following modifications:[2] 1) the internal standard (myo-inositol) was added with the last methanol addition; 2) prior to the addition of N-methylimidazole, a drop of water from a disposable pasteur pipet was added and thoroughly mixed with the contents of each tube; 3) samples were allowed to acetylate for 10 min in a boiling water bath (95 C); and, 4) the chloroform layer containing the acetylated alditols in each tube was removed and the remaining solution was further partitioned with three washes of chloroform (1 ml). The chloroform extractions were combined, dried under a stream of air (2 psi), and just prior to injection into the gas chromatograph, 200 ul of acetone were added. Three ul samples were injected into a Hewlett Packard 5830 A Gas Chromatograph equipped with a four foot column (1/8 inch I.D.), packed with 3% SP-2340 on Suppelcoport 1-1863 (100/200 mesh). The column temperature was held at 200 C for 5 min and programmed to rise 5 C/min to 240 C and maintain 240 C for 5 minutes. Injector and detector temperatures were held at 250 C. The nitrogen carrier gas flow was 25 ml/min.

[2] The first three modifications were communications from Dr. A.S. Bittner, Research Assoc. Utah State Univ. The fourth modification was a communication from Dr. A.J. Anderson, Assoc. Professor, Utah State Univ.

Two additional 200 ul aliquots for each time period were analyzed colorimetrically for their uronic acid content, using the method of Blumenkrantz and Asboe-Hansen (1973). The method is based upon the formation of a chromogen after addition of m-hydroxydiphenyl to a solution of uronic acid that has been heated to 100 C in a concentrated sulfuric acid/tetraborate solution for five minutes. The pink color is read at 520 nm.

The residue remaining in each tube after acid hydrolysis was filtered through a pre-weighed, 15 ml, sintered glass funnel (coarse porosity) and rinsed with distilled water. The residue was dried to a constant weight in a 105 C oven and ashed at 450 C in a muffle furnace for 15 hours. Lignin was determined by the difference in weight before and after ashing (Adams, 1965).

The estimation of starch was required to determine whether the hydrolysis product of starch was inflating the glucose values obtained after acetylation and quantification with the gas chromatograph. The cell walls (CMIR) were stained with iodine potassium iodide, IKI (Jensen, 1962) and compared with the color development of pea cell walls and pure corn-starch. Development revealed a very small quantity of positively stained granules. This positive staining reaction is not quantitative nor specific for starch, as xyloglucan polymers may also stain a blue/black (Van Soest, 1982). Therefore, six, 50 mg samples of the CMIR were weighed into 16 X 125 mm test tubes and approximately 0.1 g of analytical sand was added. The tubes were autoclaved for 1 hour at 120 C and 15 psi. The sterilized samples were ground against the analytical sand in sodium citrate buffer (pH 4.5) and boiled for four hours to thicken any amylose present.

Purified amyloglucosidase (Sigma A-7255, 1,4-α-D-Glucan glucohydrolase E.C. 3.2.1.3 from Rhizopus spp.) was added and the test tube was incubated for 15 hours (MacRae, 1971). An aliquot was deproteinized (Nelson, 1944) and analyzed for the presence of glucose using the glucose oxidase peroxidase system (Kilburn and Taylor, 1969). The results were negative; the bracteoles were assumed free from starch, thereon.

The above procedures accomplish the first two objectives of the study. The third objective: to biologically degrade the cell wall, was attempted on lignified and delignified CMIR. The delignification was effected with acidic sodium chlorite. A 2.0 g sample of CMIR was weighed into a 60 ml sintered glass crucible (coarse). The crucible was placed in a beaker containing approximately 100 ml of 1% acetic acid (70 C). After 10 min, 10 ml of 2% sodium chlorite was added. The solution was stirred with a glass rod and the beaker was covered with a watch glass. The oxidation proceeded for 1.5 hours, after which time, the crucible was placed under suction with a water aspirator and the sample was washed with 500 ml of hot acetic acid (1%) and 1000 ml of hot distilled water (a modification of Collings and Yokoyama, 1979, from Bittner, 1980). The loss in weight due to the solubilization of lignin was noted and the delignified cell walls were used in the enzyme hydrolysis studies, referred to as delignified CMIR.

Only readily available, reasonably priced, commercial enzyme preparations from Sigma were employed, Table 3. Preliminary incubations with cellulase sold by Sigma (C-4137) precluded its use, since its activity on carboxymethyl cellulose and solka floc was minimal. Each enzyme was incubated with its preferred substrate. Tables 4, 5

and 6 describe the contents of the incubation tubes. All of the incubations were carried out in screw-capped test-tubes that were constantly rotated. After incubation, a 1 ml aliquot was deproteinized (Nelson, 1944) and analyzed for product formation. The product of

Table 3. A listing of the enzymes employed in degradation studies.

Enzyme	E.C. Number	Source
Polygalacturonase	3.2.1.15	Sigma P-5146, Aspergillus niger
Cellulase	3.2.1.4	Calbiochem-Behring Corp. 219466 Trichoderma viride (Cellulysin)
B-Glucosidase	3.2.1.21	Calbiochem-Behring Corp. 346801 almond emulsion
Crude-Hemicellulase[a]	--	Sigma H-2125, Aspergillus niger

[a]This preparation is impure and therefore has no enzyme classification number (E.C. number).

Table 4. The contents of incubation tubes for polygalacturonase and 0.5% polygalacturonic acid (PGA) at room temperature.

	Tubes[a]		
Contents	Enzyme Control	Substrate Control	Enzyme & Substrate Experimental
pH 4.0 (0.2 M $NaC_2H_3O_2$ buffer)	10.0 ml	5.0 ml	5.0 ml
Polygalacturonase 6.5 mg/ml	0.5 ml	--	0.5 ml
1% PGA	--	5.0 ml	5.0 ml
Distilled H_2O	--	0.5 ml	--

[a]All tubes were run in triplicate. All tubes contained 10 mg of Sigma PEN-NA, benzylpenicillin sodium salt.

Table 5. The contents of incubation tubes for cellulase and B-glucosidase with solka floc[a] at 37°C.

Contents	Tubes[b] Enzyme Control	Substrate Control	Enzyme & Substrate Experimental
pH 5.0 (0.2 M $NaC_2H_3O_2$ buffer)	8.0 ml	11.0 ml	8.0 ml
B-Glucosidase 1 mg/ml &	1.0 ml	--	1.0 ml
Cellulase 4 mg/ml	2.0 ml	--	2.0 ml
Solka Floc[a]	--	25.0 mg	25.0 mg

[a]Solka floc contained 7.0% hemicellulose, 86.6% cellulose and 0.4% lignin. The solka floc was rinsed with boiling water to remove any carbohydrate contaminants.

[b]All tubes were run in triplicate. All tubes contained 10 mg of Sigma PEN-NA, Benzylpenicillin sodium salt.

Table 6. The contents of incubation tubes for crude-hemicellulase[a] with 0.25% galactomannan from locust bean gum, at 37°C.

Contents	Tubes[b] Enzyme Control	Substrate Control	Enzyme & Substrate Experimental
pH 5.5 (0.2 M $NaC_2H_3O_2$ buffer)	5.0 ml	5.0 ml	--
Crude-Hemicellulase 150 mg/ml	5.0 ml	--	5.0 ml
0.5% Galactomannan	--	5.0 ml	5.0 ml

[a]10 ml of hemicellulase (300 mg/ml) were dialyzed against running cold tap water. The final concentration was 150 mg/ml.

[b]All tubes were run in triplicate. All tubes contained 10 mg of Sigma PEN-NA, Benzylpenicillin sodium salt.

polygalacturonase, galacturonic acid, and the products of hemicellulase, predominantly galactose, were analyzed with the cupric sulfate reduction method of Nelson (1944), Figure 2. The analysis of cellulase and B-glucosidase product, glucose, was performed with the glucose oxidase peroxidase system (Kilburn and Taylor, 1969), Figure 3. The tubes were monitored over time until the production of reducing sugars remained constant, Figures 4, 5 and 6.

The enzyme activity curves provided information concerning the length of time required for maximal hydrolysis of ideal substrate and the quantity of product formed from ideal substrate. The time periods for incubation with CMIR and delignified CMIR were extended in an attempt to alleviate the problems of enzyme/substrate contact. Polygalacturonase incubations were allowed to continue for approximately 5 hours. All other incubations lasted for five days. Fifty mg samples of CMIR and delignified CMIR were weighed into 16 X 125 mm screw-capped tubes. Approximately 0.1 g of analytical sand was added and the tubes were autoclaved for 1 hour at 246 C and 15 psi. For each of the enzyme treatments: six experimental, one enzyme control and one cell wall control were run. The following procedure applies for all treatments: 0.5 ml buffer was added; the sample was ground against the sand in the tube with a glass rod; the rod was rinsed with a known volume of buffer and removed; the final volume was 5 ml. The conditions of incubation (pH, temperature) were the same as those described previously in Tables 4, 5 and 6, with these exceptions: The concentration of penicillin was reduced to 1 mg. The concentration of polygalacturonase was increased to 1 ml (15 mg/ml).

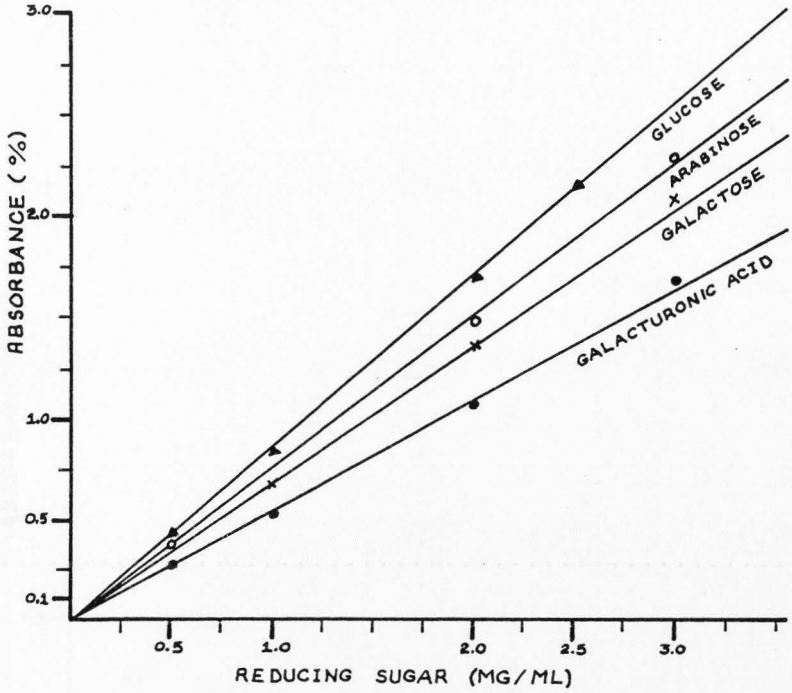


Figure 2. Standard curves of monosaccharides after deproteinization and analysis by the reducing group assay. The concentrations listed correspond to the standard solutions prior to the 1:20, 1:3 dilutions involved in deproteinization and analysis.

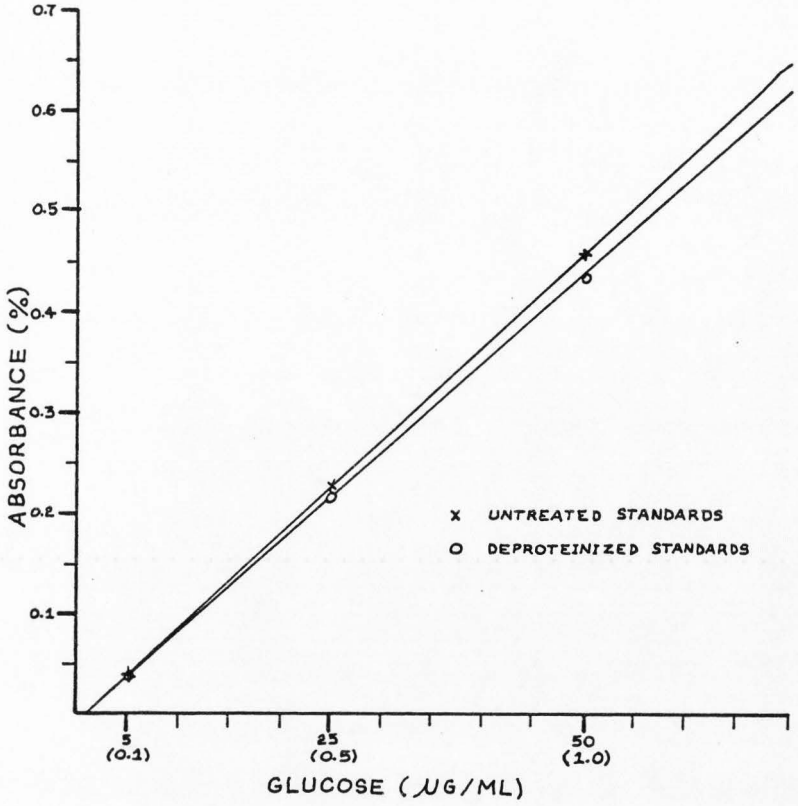


Figure 3. Standard curves of glucose solutions analyzed by the glucose oxidase peroxidase procedure. Untreated standards were prepared in ug/ml. Standards prepared in mg/ml (listed in parentheses) were deproteinized prior to analysis. The dilution factor was (1:20)(1:5).

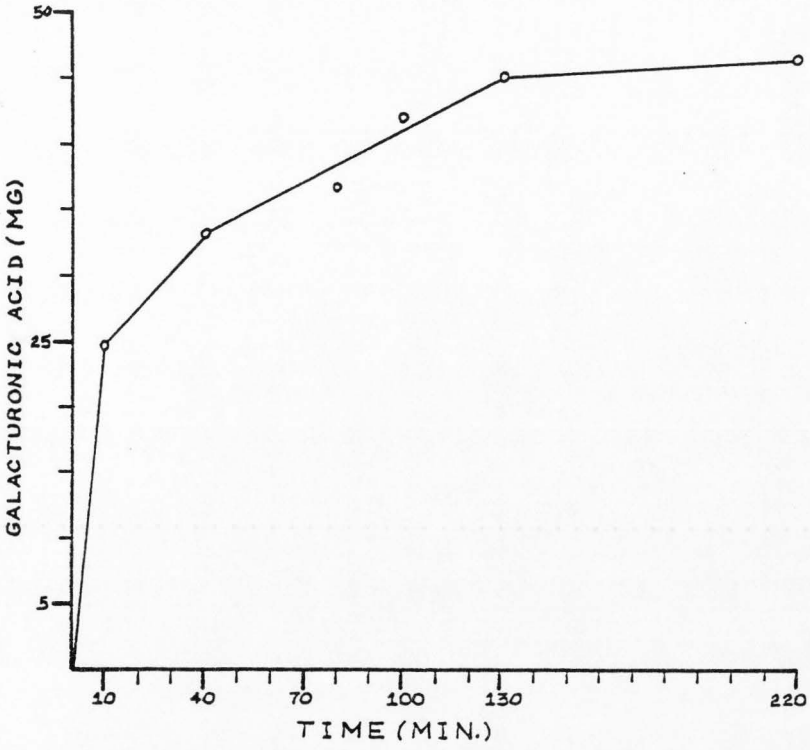


Figure 4. Activity curve of polygalacturonase (20.06 units, 3.4 mg) and 0.5% polygalacturonic acid, 25 C, pH 4.0 (0.2 M $NaC_2H_3O_2$). Maximum hydrolysis was obtained after approximately 4 hours with 81% hydrolysis of the substrate. Activity was monitored with the reducing group assay.

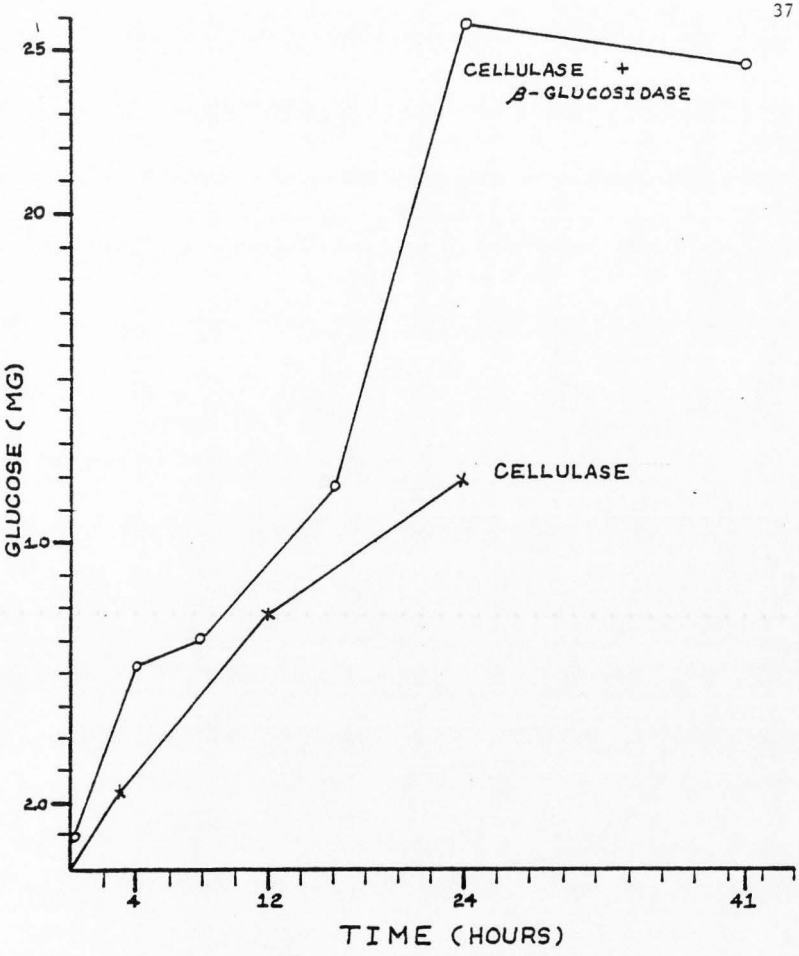


Figure 5. Activity curve of cellulase (40 units, 4 mg) and cellulase (80 units, 8 mg) with the addition of B-glucosidase (5.1 units, 1 mg) with solka floc substrate, 37 C, pH 5.0 (0.2 M $NaC_2H_3O_2$). Maximum hydrolysis was reached after 24 hours with 93% degradation of solka floc. Activity was monitored with the glucose oxidase peroxidase assay.

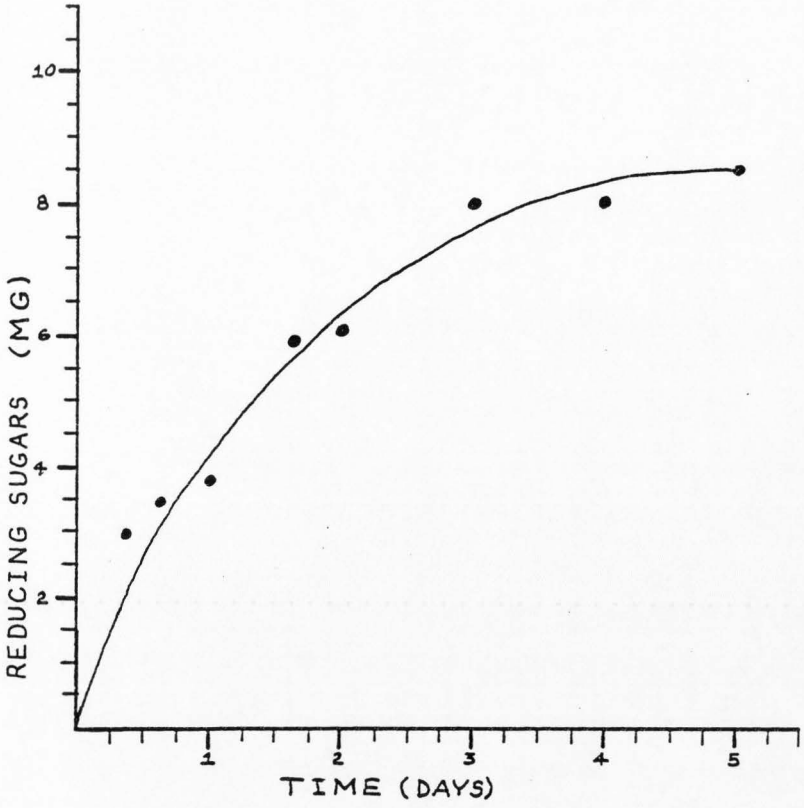


Figure 6. Activity curve of crude-hemicellulase (53.5 units, 675mg) with 0.25% galactomannan, 37 C, pH 5.5 (0.2 M $NaC_2H_3O_2$). Maximum hydrolysis obtained after 5 days with 30.6% degradation of galactomannan. Activity was monitored with the reducing group assay, using galactose standards.

After the incubation period was completed, the tubes were centrifuged and a 1 ml aliquot was deproteinized and analyzed for product formation. The remaining 4 mls of solution were used for the sequential treatment with pectinase and/or cellulase and B-Glucosidase. The pH was adjusted for optimum activity of the second enzyme upon its addition. Table 7 clarifies the quantity of solutions added. The tubes were incubated for the length of time required for

Table 7. Additions to the enzyme/substrate tubes for the sequential enzyme treatments.

	Enzyme/Substrate Tube		
Solutions	Cellulase B-Glucosidase	Crude Hemicellulase	Polygalacturonase
0.2 M $C_2H_4O_2$	5.0 ml	8.0 ml	--
0.2 M Na $C_2H_3O_2$	--	--	4.0 ml
Polygalacturonase 15 mg/ml	1.0 ml	1.0 ml	--
Cellulase 4 mg/ml	--	--	2.0 ml
B-Glucosidase 1 mg/ml	--	--	1.0 ml
TOTAL volume	10 ml	13 ml	11 ml

the newly added enzyme and analyzed for product formation as described. Concentrations of product were adjusted for the interference in absorbance caused by the enzyme proteins and the delignified CMIR, no interference was noted from the lignified CMIR.

The statistical analysis of glucose oxidase peroxidase data included a multiple mean comparison and a pairwise mean comparison

using single factor (glucose production) analysis of variance. Statistical analysis of the total reducing group data was performed with two-factor (reducing groups and delignification) analysis of variance as well as multiple mean contrasts (Neter and Wasserman, 1974).

Germination trials

The final objective of this project was to assess the effectiveness of the biological degradation technique on intact Atriplex gardneri seeds. The most effective enzyme treatment on delignified CMIR was applied to delignified seeds. Delignification followed the method described earlier for the CMIR. After preliminary trials, the tolerance of the seed to the delignification reagent and to the delignification temperature was doubted. Therefore, a second series of delignification treatments were run at 35 C. To test for tolerance to the acid chlorite, a blank was heated in acetic acid without the addition of oxidizing agent. A comparison of the experimental technique with several of the more common germination techniques ensued (Table 8). For each treatment,

Table 8. A description of the comparative germination pre-treatments.

Treatment Applied	Treatment Description
Surface Sterilization	5 min in 5% chlorox, 15 min. tap rinse
Physical Scarification	above followed by 5 rounds through a sand-paper hammermill, after drying
Tap Water Leach	2 hours in running, cold tap water
Boiling Water Leach	2 min in boiling water
Acid Scarification	30 min in 72% H_2SO_4, 30 min tap rinse

400 seeds were planted. The seeds were treated and allowed to air dry for one week before being sown into a sandy loam soil in glass petri dishes, 25 seeds per dish. The soil and petri dishes had been autoclaved. The soil was allowed to ventilate in a sterile environment for five days before use (USDA, 1952). The petri dishes were placed in growth chambers regulated at 20 C day (15 hours) and 15 C night (9 hours). The soil was kept friable by the addition of approximately 5 ml of water per week. Germination was followed weekly for one month. Only healthy seedlings were counted. Those seedlings with poorly developed roots (less than 1.5 cm) or poorly developed shoots (less than 2 cm, curled cotyledons, unhealthy purplish tinge) were not counted. The percentage germination of each treatment was reported on the basis of the total population planted, and the estimated number of filled seeds within that population (preliminary data). The estimated fill was 38%.

RESULTS AND DISCUSSION

The objectives of this study were to detail the major components of the Atriplex bracteole cell wall and to attempt degradation of the wall using inexpensive, easily obtainable commercial enzyme preparations, with the development of a seed pre-treatment method in mind. The data collected indicate that the rigidity of the bracteoles is due to secondary thickening with the deposition of large amounts of hemicellulosic polymers and lignin. Enzyme degradation of this cell wall can be achieved only after delignification. The development of a pre-treatment method will require further research on the tolerance of intact seeds to the delignification process.

Atriplex cell walls were isolated as the chloroform:methanol insoluble residue (CMIR). The two-step acid hydrolysis of Bittner et al.(1980), revealed the major components of the cell wall polymers. The yield of neutral sugars released from the sample residue by the sulfuric acid hydrolysis was monitored at various time intervals (Figures 7 and 8) using gas liquid chromatography of their alditol acetates. The yield of acidic sugars was monitored over time (Figure 9) using the meta-hydroxydiphenyl colorimetric reaction. Neutral sugars monitored included rhamnose and fucose, although neither of these sugars were found in the hydrolysate. Maximum yields of xylose, arabinose, and galactose were reached after 30 to 45 minutes. The maximum yield of glucose was obtained after 3 hours. Maximum uronic acid yields were obtained after 1 hour. These results

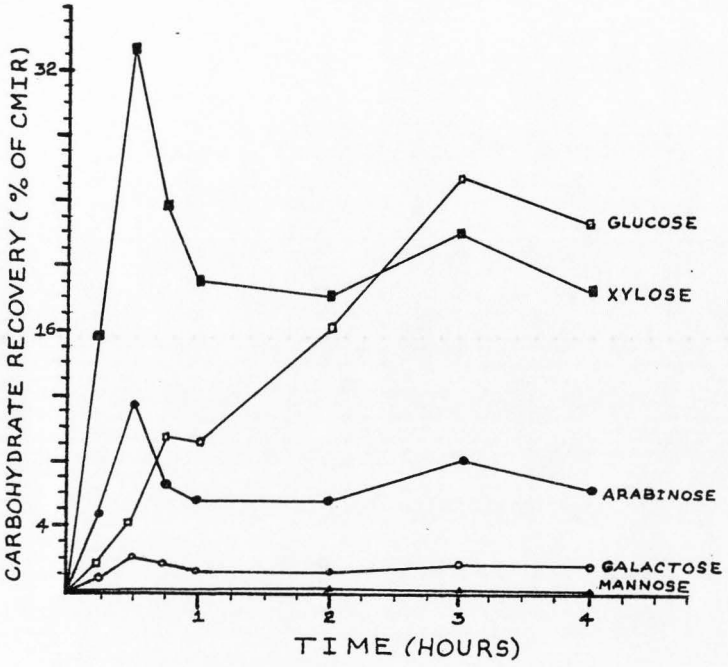


Figure 7. Monosaccharide yield from the CMIR during the four hours of hydrolysis in 2 N H_2SO_4, Sept. 26, 81. Yields were quantified using gas chromatography of the acetylated alditols.

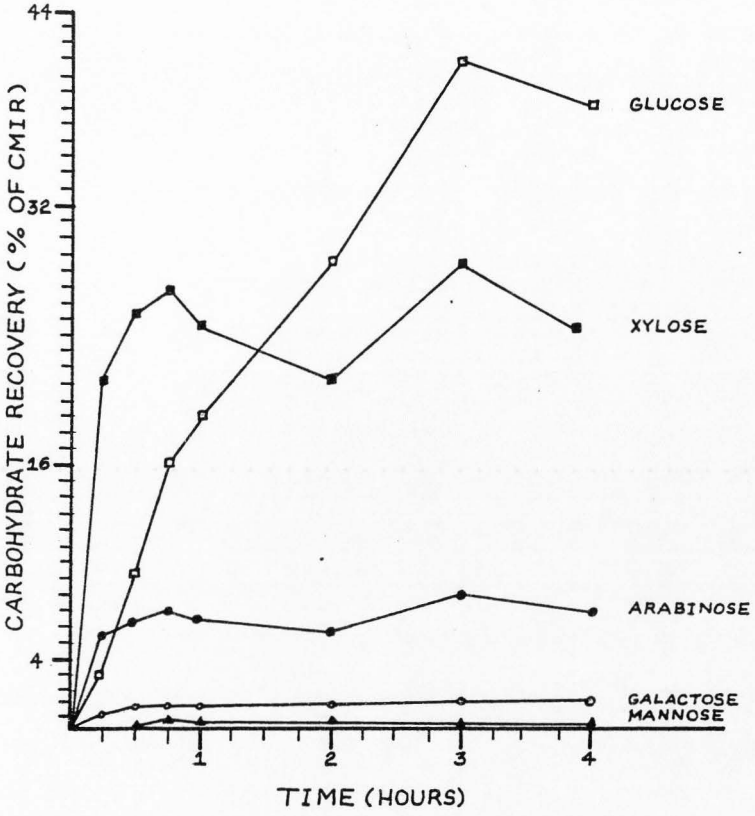


Figure 8. Monosaccharide yield from the CMIR during the four hours of hydrolysis in 2 N H_2SO_4, Dec 9, 81. Yields were quantified using gas chromatography of the acetylated alditols.

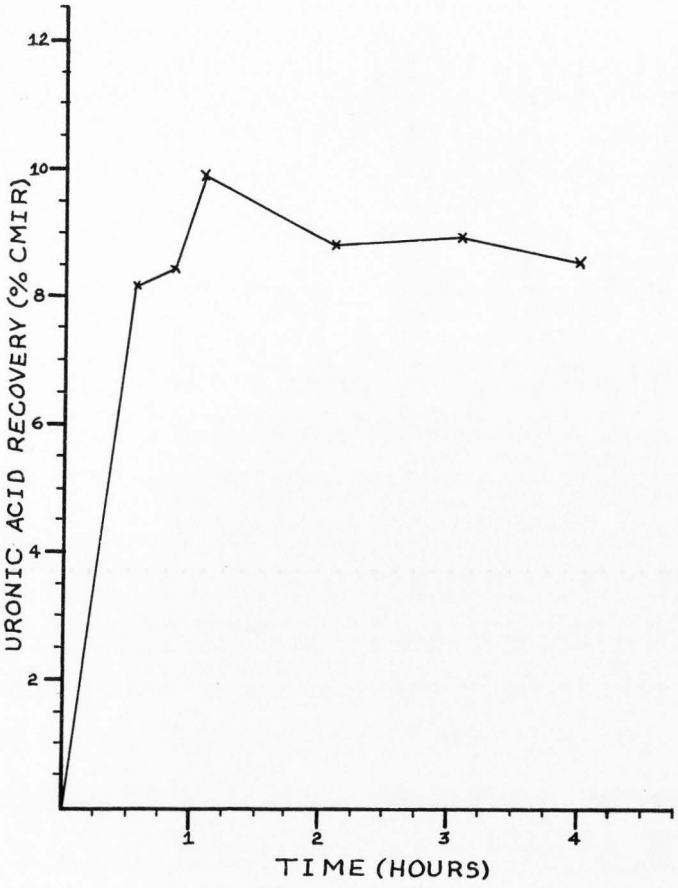


Figure 9. Uronic acid yield from the CMIR during the four hours of hydrolysis in 2 N H_2SO_4, analyzed with meta-hydroxydiphenyl.

agree with those of Bittner et al.(1980). The 3 hour time period was chosen for the remaining samples to maximize glucose recovery and a correction factor was used to adjust the three hour values of xylose, arabinose, and galactose, as well as uronic acids for the remaining samples (Table 9). Carbohydrate yields are reported as a percentage of the total dry weight of the CMIR in Table 10 and total sugars, crude-lignin values, xylose:glucose, xylose:arabinose, and xylose: uronic acid ratios are reported in Table 11, again as a percentage of the total dry weight of the CMIR.

Table 9. Three hour acid hydrolysis yield correction factors.

Carbohydrate X	Factor
Arabinose	1.38
Xylose	1.50
Galactose	1.10
Uronic Acid	1.09

In the mature Atriplex bracteoles, the middle lamella and primary cell walls are a small fraction of the total dry weight of the cell walls. This may be the reason for the lack of rhamnose and the very low arabinose, galactose and uronic acid contents, usually associated with the pectin fraction of the cell wall. Additional loss of arabinose may have occurred naturally with the drying of the bracteoles, as furanosidic sugars are most susceptible to the formation of Maillard reaction products (Van Soest, 1982).

The fact that hemicellulose content may approach that of cellulose in lignified tissues has been established (Brice and Morrison, 1982; Bittner, 1980). Approximately equal yields of glucose

Table 10. Carbohydrate yields after two-step sulfuric acid hydrolysis. Yields are an average of three sub-samples and are reported as a percentage of the CMIR dry weight. Samples were hydrolyzed by 72% H_2SO_4 for 30 min, and then diluted to 2 N for 3 hours hydrolysis at 95 C. Neutral sugars were quantified by gas chromatography of the acetylated alditols. Acidic sugars were quantified by colorimetry with m-hydroxydiphenyl.

Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic Acid
7.2	30.4	0.3	1.2	28.6	9.5
7.3	29.2	0.3	1.1	28.4	--
7.1	26.8	0.5	1.1	41.3	8.7
		Overall Average			
7.2	28.8	0.4	1.1	32.8	9.1

Table 11. Total sugars, crude-lignin, xylose:glucose (X/G), xylose: arabinose (X/A), xylose:uronic acid (X/U) ratios after two-step acid hydrolysis. Yields are an average of three sub-samples and are reported as a percentage of the CMIR dry weight. Samples were hydrolyzed by 72% H_2SO_4 for 30 min, and then diluted to 2 N for 3 hours hydrolysis at 95 C. Neutral sugar yields were quantified by gas chromatography of the acetylated alditols. Lignin was recovered as the acid insoluble residue.

Total Sugars	X/G	X/A	X/U	Crude Lignin
77.0	1.1	4.2	3.2	16.8
66.2	1.0	4.0	--	20.7
76.9	0.7	3.8	3.1	13.6
		Overall Average		
73.4	0.9	4.0	3.2	17.0

and xylose reveal that both glucans and xylans figure prominently in the Atriplex cell wall structure. Since cellulosic and non-cellulosic glucose are not differentiated by the method, the origin of the glucose polymers can only be hypothesized. Therefore, in actuality, hemicellulosic compounds may exceed cellulosic compounds in the Atriplex cell walls. An estimate of the non-cellulosic glucose can be inferred by the initial glucose peak after the first hour of hydrolysis (Figures 7 and 8), approximately one-third of the glucose yield. Hemicellulosic glucan is more available to the mineral acid, by virtue of its linkage and structure, and should, theoretically be hydrolyzed first. The likely forms of hemicellulosic glucose are B-1,3-glucan, B-1,4-glucan and xyloglucan. (Mixed B-1,3 and B-1,4-linked glucans are not common in dicots (McNeil et al. 1979).

The presence of a xyloglucan in the bracteoles is practically assured by the positive, blue-black staining reaction with IKI (Van Soest, 1982) and a lack of starch indicated by purified α-amylase assay. The xylose:glucose ratios commonly reported for xyloglucan are greater than 0.5 (McNeil et al. 1979). The xylose:glucose ratio reported in Table 11 is an average of 0.91, but this ratio does not reflect the X/G relationship within a purified polymer. The Atriplex xyloglucan polymer may be substituted with arabinose or galactose residues, but no fucosyl residues were isolated from the hydrolysate.

Xylan polymers form the greatest proportion of hemicellulose in angiosperms (Aspinall, 1980). No doubt, the xylose yield from Atriplex bracteoles predominantly originates from a linear B-1,4-linked xylan polymer. The most common substituent in dicot xylans

is 4-O-methyl-D-glucuronic acid (Aspinall, 1980). Atriplex bracteoles contain an average of 9.09% uronic acids that may be primarily, but not totally attributable to xylan polymers. Other possible branching substituents are arabinose and/or galactose. Branching in Atriplex xylan is at a minimum, however, given the high X/A, X/G, and X/U ratios (Table 11). The degree of linearity is indicative of the stage of maturity and is often associated with increased lignification (Bittner, 1980).

Although glucomannans (linear B-1,4-linked mannose) form the bulk of hemicellulose in the secondary walls of gymnosperms, only small amounts (3-5%) are common in angiosperms (Northcote, 1972). Mannose, 0.35%, was hydrolyzed from the Atriplex bracteoles. Such a small percentage may reflect a small quantity of B-1,4-linked mannan or may be an artifact of the hydrolysis procedure, through the acid-catalyzed transformations of fructose and/or glucose (Shallenberger and Birch, 1975).

The cellulosic glucose is that portion of the total glucose yield that is least readily hydrolyzable. As such, it is represented by the second peak for glucose in Figures 7 and 8, approximately two-thirds of the total yield. The accessibility and crystallinity of the cellulose fibrils affect the extent of hydrolysis by acid. Physical shielding by lignin polymers and/or the presence of acid-insoluble lignin-carbohydrate complexes may significantly lower the recovery of cellulose as its glucose monomer (Millit et al. 1979; Philipp et al. 1979). The delignification of plant cell walls low in lignin has been shown to be an unnecessary step prior to acid

hydrolysis (Bittner et al. 1980). But, the quantity of acid insoluble residue obtained from Atriplex indicates that the bracteoles are thoroughly lignified and recovery of polysaccharides was limited.

Crude-lignin is reported as approximately 17.0% of the Atriplex bracteoles. By way of comparison, rice hulls contain approximately 19%, sycamore wood contains 22%, and redwood contains approximately 34% lignin (Rosenberg, 1980). The value reported here may underestimate the actual lignin content due to the loss of acid-soluble lignin (Van Soest, 1982). In angiosperm woods, treatment with 72% sulfuric acid for two hours (Klason method) will dissolve approximately 25 to 60% of the total lignin (Lai and Sarkanen, 1971). Following dilution with water to 3% H_2SO_4 and hydrolysis at the boiling point for 4 hours, most, but not all, of the lignin will re-precipitate.

On the other hand, the method probably overestimates the lignin content due to the initial presence of acid-insoluble lignin-carbohydrate complexes, cutin and Maillard reaction products (Van Soest, 1982) and the acid-catalyzed condensation of lignin with non-lignin polyphenols (tannins) and protein (Lai and Sarkanen, 1971). Although, the protein content of herbaceous plants decreases with maturity, an estimate of co-condensed protein is 8-12% for the mature plant (Lai and Sarkanen, 1971). Crude-lignin values are usually corrected with a factor equal to 6.25 X the total nitrogen content. Alternatively, the plant sample should be extracted with neutral detergents that form strong, soluble protein complexes (Van Soest, 1982) prior to Klason lignin determination. A preferable method of

lignin determination is the extraction of the plant sample with thioglycolic acid in 2 N hydrochloric acid producing lignin thioglycolic acids that are free of polyphenol and protein impurities (Lai and Sarkanen, 1971).

Enzymolysis of the cell wall

Enzyme studies were designed to indicate the extent of degradation possible with and without delignification of the bracteoles, and to explore the possibility of synergism with sequential additions of enzymes. In all cases, delignification dramatically increased the ability of enzymes to degrade the cell wall, reflecting the degree of physical encrustation and chemical association between lignin and hemicellulosic and cellulosic polymers.

The yields of glucose obtained from cellulase incubations with lignified and delignified cell walls are reported in Table 12. Conspicuously missing from this table are the lignified/cellulase and B-glucosidase[3] followed by polygalacturonase; and the delignified/polygalacturonase followed by cellulase and B-glucosidase treatments. These treatments were analyzed by the total reducing group assay of Nelson (1944) and are included in Table 14.

A multiple mean comparison provides evidence for the superior action of cellulase on delignified cell walls (Table 13), yielding significantly greater quantities of glucose than all other treatments. Pairwise comparison of the means through single factor analysis of

[3]This treatment will be referred to simply as, cellulase.

Table 12. The yield of glucose obtained after enzymolysis of lignified and delignified cell walls.

Treatment	Treatment Mean (%)[a]	S.D.
Lignified Cell Wall		
Cellulase/B-Glucosidase	4.9	0.6
Polygalacturonase & Cellulase/B-Glucosidase	8.3	0.3
Delignified Cell Wall		
Cellulase/B-Glucosidase	24.2	3.1
Cellulase/B-Glucosidase & Polygalacturonase	19.8	1.6

[a]Six repetitions were included in each treatment. Values were obtained from the glucose oxidase peroxidase assay and reported as a percentage of the CMIR dry weight.

variance revealed that the incubation of delignified cell walls with cellulase produced a 16 - 22% greater yield of glucose than the lignified/cellulase treatment. Differences between treatments within the lignified and delignified blocks were significant, but not substantial. A pre-treatment with polygalacturonase appears to improve the glucose yield due to cellulase activity on lignified cell walls, but the degree of improvement is rather small as can be seen from the confidence interval.

Treatments that were analyzed by the total reducing group assay (Table 14) also showed a definite yield improvement upon delignification. The most dramatic improvement being the activity of the hemicellulase enzyme. The treatments and delignification effects were compared using two-factor analysis of variance (Table 15). The analysis revealed no interactive, synergism between enzyme treatments and delignification;

Table 13. Lignified and delignified cell wall cellulase treatments: Analysis of treatment means obtained by the glucose oxidase peroxidase assay.

Comparison	Treatments[a] Compared	Test Statistic	Rejection Region	95% Level Confidence Interval
Multiple Mean	L-C	0.5211		
	L-P & C	0.1121	F = 5.80	
	D-C	15.8988		
	D-C & P	3.4680		
Pairwise Mean	L-C vs D-C	219.008	F = 4.96	16.3 - 22.1 %
	L-C vs L-P & C	142.82	F = 4.96	2.72 - 3.96 %
	D-C vs D-C & P	9.1355	F = 4.96	1.14 - 7.52 %

[a]L-C = Lignified cell wall, cellulase treatment

L-P & C = Lignified cell wall, Polygalacturonase followed by celluase

D-C = Delignified cell wall, cellulase treatment

D-C & P = Delignified cell wall, cellulase followed by polygalacturonase

Table 14. Comparative degradation of lignified and delignified Atriplex cell walls incubated with enzymes singularly and in sequence.

Enzyme[a] Treatment	Degradation[b] (%) Lignified	S.D.	Delignified	S.D.
C	...		35.0	3.0
H	0.7	1.1	24.8	3.5
P	8.8	1.6	35.7	2.1
C & P	14.0	2.9	35.8	3.3
P & C	...		16.0	0.9
H & P	11.6	2.9	37.3	3.4

[a]Only treatments analyzed by the reducing group assay are included. H = Hemicellulase, P = Polygalacturonase, C = Cellulase and B-Glucosidase, & = Sequential Treatment.

[b]Degradation is reported as a percentage of the CMIR dry weight. All values are the means of six samples.

although, significant differences were noted among enzyme treatments and between lignified and delignified cell walls. Hence, multiple contrasts (Table 16) for the factor level means were worked out. The difference between lignified and delignified treatment means revealed that delignified cell walls as a whole exceeded lignified cell walls in their capacity to be degraded by an interval of 19.37 to 29.89%. Among the enzymes, the only inferior treatment proved to be hemicellulase, singly. Hemicellulase treatments were 0.08 to 21.59% less effective than all other treatments, including the sequential hemicellulase treatment, H & P. Since no interactive effects were noted, the action of combination treatments must be simply additive.

Table 15. Two-factor analysis of variance for enzyme treatments and delignification of the Atriplex cell walls.

Source of Variation[a]	Sum Squares	df	Mean Square	F Statistic	95% level Rejection Region
Between Treatments	7,391.3840	7	1,055.91		
Enzyme Treatments	1,206.9321	3	402.31	4.9868	2.8666
Delignification Effect	6,098.6479	1	6,098.6479	75.5954	2.8666
Interactions	85.8040	3	28.60	0.3545	4.1133
Error	3,226.9960	40	80.6749		
Total	10,618.3800	47			

[a]The enzyme treatments included in this analysis are those that were monitored by the total reducing group assay: hemicellulase, polygalacturonase, cellulase & polygalacturonase and hemicellulase & polygalacturonase. The treatments included were followed on lignified and delignified cell wall (Table 14).

Table 16. Multiple contrasts between lignified and delignified treatments and among enzyme treatments that were analyzed with the reducing group assay.

Contrast	Estimated Difference (%)	95% level Confidence Interval (%)
All lignified vs all delignified	24.6	19.4 to 29.9
Between delignified treatments:[a]		
P vs H	10.8	0.1 to 21.6
C & P vs P	0.2	-10.6 to 10.9
H & P vs C & P	1.5	- 9.3 to 12.2

[a] H = Hemicellulase, P = Polygalacturonase, C = Cellulase and B-Glucosidase, & = Sequential treatment.

The treatment means for cellulase, and polygalacturonase followed by cellulase were omitted from the two-factor ANOVA table due to the missing reducing group data for lignified treatments (Table 14). However, comparisons between the delignified cellulase mean, the polygalacturonase mean, the cellulase & polygalacturonase mean, and the hemicellulase & polygalacturonase mean reveal that all are equally effective at the production of reducing groups. Several interesting observations should be made. First, in direct opposition to the observations of the effectiveness of the Polygalacturonase & Cellulase treatment for lignified cell walls (Table 12), the effectiveness of the polygalacturonase pre-treatment is diminished on delignified cell walls (Table 14). In table 14, the values for polygalacturonase and polygalacturonase & cellulase were obtained from aliquots of the same sample, so that, one would expect equal

values even if the cellulase addition provided no additional reducing groups. The small standard deviation recorded for the polygalacturonase & cellulase treatments does not suggest bacterial contamination as the cause of the decrease in free reducing groups. Is the polygalacturonase producing a product from the delignified cell wall that is non-competitively inhibiting the action of cellulase? This inhibition was not noted when the polygalacturonase addition followed the cellulase components on delignified cell wall, as measured by the reducing group assay. When the polygalacturonase followed cellulase, an inhibiting affect would be obscured by the cellulase product already in solution.

Table 17. A comparison between the values obtained from glucose oxidase peroxidase (G.O.P.) and the total reducing group (T.R.G.) assays.

Delignified Cell Wall Treatment	% Degradation[a]			
	G.O.P	S.D.[a]	T.R.G.	S.D.
Cellulase	24.2	3.1	35.0	3.0
Cellulase & Polygalacturonase	19.8	1.6	35.8	3.3

[a]Degradation is reported as a percentage of the CMIR dry weight. All values are the means of six samples.

A comparison between the two assays (Table 17) was available from the results of two of the delignified treatments. The values reported in Table 17 represent aliquots from the same samples. The larger quantity of reducing groups as compared with glucose may reflect an impurity in the cellulase (cellulysin) or B-Glucosidase

preparations. The 10% difference may be due to the stereo-specificity of the glucose oxidase reagent, as α-glucose is not oxidized by glucose oxidase (Caraway, 1976). Or, the difference may imply the presence of other monosaccharides in solution (or both). The latter possibility reflects an impurity in the cellulase preparation. Even a highly purified cellulase preparation contains some xylosidase activity (Sinner et al. 1979; Dewey and Mandels,1980). That the difference is not due to interference by amino acid groups is certain, since a deproteinization step was included in the procedure.

Apparently, the only pre-treatment necessary for optimal enzyme catalysis is delignification. The yield of glucose obtained after incubation of delignified substrate with cellulase enzymes approached that obtained from acid hydrolysis: 24% glucose after enzyme incubation compared with 32.8% after acid hydrolysis. A similar relationship between the enzyme and acid hydrolyzable cellulose has been noted for delignified Sprucewood: 30% after enzymolysis and 46% after total acid hydrolysis (Sinner et al. 1979).

Restrictions to the extent of degradation by cellulase are numerous. Cellulase enzymes are known to be inhibited by excess product (cellobiose and glucose) in solution (Wood and McCrae,1979; Humphrey,1979) and by the accumulation of gluconolactone (Dewey and Mandels,1980). Lactone structures are formed by the oxidation of aldoses by mild reducing agents, usually a five-membered ring is formed (Shallenberger and Birch,1975). If the yield of glucose was limited by competitive inhibition with the above compounds, the addition of fresh enzyme and the removal of hydrolysate would allow

further degradation to occur.

The impervious nature of the crytalline substrate and associated polymers not only restricts entry of the enzyme and access to the glycosidic bonds, but also, release of the hydrolyzed oligosaccharides is limited (Gritzali and Brown,1979). Inhibition of the cellulase enzyme is known to occur in the presence of phenolics (Griffiths, 1981) which may have been released within the structure of the cell wall after acid chlorite treatment (Brice and Morrison,1982). In fact, a browning of the delignified sample after autoclaving, but not of the lignified sample, indicates that delignification released bound monosaccharides, most probably xylose (Shallenberger and Birch,1975). The heat of the autoclave (120C) could then trigger carmelization or Maillard reactions to occur. After buffer solutions were added, an increase in the reducing groups was noted (Table 18), and a pinkish color developed, especially with the addition of pH 4.0 sodium acetate. The tremendous increase in the catalyzing power of hemicellulase is probably directly related to the solubilization of its substrate in buffer solution after delignification. An unusually large increase in hemicellulase activity after delignification has been noted in studies on Digitaria decumbens (Pangola grass) (Ford,1978).

The activity of a commercial endopolygalacturonase enzyme on Atriplex cell walls is surprising, due to the supposed lack of adequate substrate (9.09% uronic acids that were assumed to be associated with lignin-carbohydrate complexes). However, like other commercial preparations, commercial pectinases are known to contain impurities (Rombouts and Pilnik,1973). The rapid increase in

Table 18. The quantity of reducing groups leached from delignified, autoclaved cell walls of Atriplex, by 0.2 M sodium acetate buffer solutions.

	% of Sample[a]		
pH	Glucose[b]	Total Reducing Groups[b]	S.D.
4.0	0	1.25	1.2
5.0	0	3.15	0.35
5.5	0	3.90	0.14

[a]Percentages reported are based on the dry weight of the delignified CMIR. Values are an average of two samples.

[b]Assays for glucose were performed using the glucose oxidase peroxidase system. Assays for total reducing groups followed Nelson (1944).

reducing groups after a one hour incubation period (Figure 4) testifies to the activity of an exo-acting enzyme rather than an endopolygalacturonase (Mill and Tuttobello, 1961). The presence of an impurity may explain the slight improvement in glucose yield when lignified cell walls were pre-treated with polygalacturonase prior to cellulase. As previously noted, exactly the opposite effect occurred with delignified cell wall.

Several enzyme treatments appeared to be approximately equal in terms of the quantity of reducing groups produced: cellulase, polygalacturonase, hemicellulase and polygalacturonase, cellulase and polygalacturonase. Of these, cellulase was chosen for treatment on intact, delignified seeds. The following reasons were included in that decision: 1) Cellulase is stable over long periods. 2) Cellulase catalyzes the hydrolysis of the most rigid structure

present in the cell wall. 3) Cellulase degraded approximately 24% of the cell wall to monosaccharides (Table 12), indicating very thorough hydrolysis.

Germination trials

The delignification of intact Atriplex seeds with acid chlorite followed by further degradation with cellulase was attempted to improve the germination percentage of the species. A comparison of the experimental technique with several of the more common germination techniques led to the conclusion that physical scarification surpasses all other treatments at increasing germination (Table 19).

The first column of Table 19 represents the proportion of germinated seeds to the number of seedcases planted. The second column is adjusted by a factor of 1/0.38 to report a percentage of germinated seeds to the actual number of embryos. The 38% fill estimate is based on the excision of several hundred seeds (preliminary data). The correlation between the percentage germination of embryos released after scarification and the adjusted percentage germination of those contained within the bracteoles after scarification attests to the accuracy of the figure.

The seed appears to be completely intolerant of temperatures above that of metabolic temperature, 37 C. The seed was left inviable after the boiling water treatment and after delignification at 70 C. This was confirmed with a tetrazolium test (Association of Official Seed Analysts, 1970). The delignification reagent was tolerated at low temperatures (35 C), also confirmed by tetrazolium

Table 19. The germination of Atriplex seeds after physical and chemical treatments.

Treatment	Germination (%)	Adjusted (%)[a]
Surface sterilized	5.5	14.5
Surface sterilized and physically scarified:		
embryos contained within bracteoles	9.6	25.3
embryos released from bracteoles	24.0	N/A
combined	28.0	N/A
Cold water leach (2 hrs)	4.0	10.5
Boiling water leach (2 min)	0	0
72% H_2SO_4 (30 min)	6.0	15.8
Delignification 70 C :		
15 min	0	0
30 min	0	0
45 min	0	0
Delignification 35 C :		
15 min	3.0	7.9
30 min	3.0	7.9
30 min blank	2.8	7.2
Cellulase treatments		
after 15 min delignification 70 C	0	0
after 30 min delignification 70 C	0	0
after 45 min delignification 70 C	0	0
after 15 min delignification 35 C	0	0
after 30 min delignification 35 C	0	0
after 30 min blank 35 C	0	0

[a]Percentage germination corrected for the estimated 38% fill of the seeds, a factor of 1/0.38 was used.

staining. The analogous values of the 15 and 30 minute seeds (treated with acidic sodium chlorite) and the 30 minute blank seeds (treated with acetic acid only) suggest that the bracteoles are not delignified in 30 minutes time. Delignification has been successfully demonstrated at mild temperatures with acid chlorite after 24 hours (Dietrichs and Zschirnt, 1972). However the tolerance of the embryo to the delignification reagent must be questioned due to the disparity between the control (surface sterilized) and the delignified seeds. It is suggested that the tolerance of the seed to the delignification reagent be tested over time to determine the maximum period of immersion in the acid chlorite.

The absence of germination after the delignified seed was incubated with cellulase was probably due to the anaerobic conditions of the incubation. Complete immersion in liquid is seldom beneficial to a seed. The abrupt rehydration causes cytological damage and leakage of cell contents as well as inhibition of metabolic pathways (Bewley and Black, 1978). Bubbling oxygen through the soaking water may only aggravate the damage to the embryo, by allowing the embryonic cells to advance to a more succeptible, oxygen-demanding stage of development (Heydecker, 1977). The Atriplex bracteoles, being heavily lignified presented a natural barrier to oxygen penetration and water permeation. Once water passed through the interstices of the bracteole, the seed became completely waterlogged. Any phenolics released within the structure of the seed case during delignification may have further prevented metabolic activity through the competitive binding of oxygen.

Treatment with 72% H_2SO_4 solubilized cellulose, but without any dramatic improvement in germination over the surface sterilized treatment. Cold water leaching dissolved NaCl, also without improvement of germination. Delignification proved to be less successful than the control, and enzymolysis was disastrous for the embryos.

The only successful treatment was physical scarification with a sandpaper hammermill. Still, physical scarification succeeded with only one-quarter of the filled seeds. The method could be refined to limit the degree of destruction to the bracteoles, so that the embryos would still be retained within the structure. When the bracteoles are present, they function to protect the cotyledons and apical meristems and to retain the cotyledons and shoot apical meristem until the radicle is fully oriented and developed. The most common problems with loose embryos were 1) weak or non-existant roots 2) purplish coloration of the stem and cotyledons and 3) curling of the embryonic leaves. These symptoms are suggestive of a nutrient or water imbalance or fungal infection, all of which may be attributable to a damaged radicle.

A healthy seedling consisted of a white tap root with many root hairs, a pale green stem and green cotyledons. The healthiest seedlings were those planted approximately 1 cm deep in the soil. Seeds that were not covered by soil had lengthy, fully developed roots that were completely disoriented, circling in the air. The Atriplex spp. may be an example of the cooperative alliance between geotropism and photosensitivity (Salisbury and Ross, 1978).

SUMMARY AND RECOMMENDATIONS

This study has provided valuable, preliminary data descriptive of the cell components of Atriplex. A computer search of the Biosis Previews file confirms that the data presented herein joins a very limited base of information on the family Chenopodiaceae (Appendix B).

Clarification of the bracteole restraint on germination and the development of an improved seed pre-treatment were the objectives of this study. Bracteole cell walls were isolated and hydrolyzed with 72% sulfuric acid followed by dilution and further hydrolysis. Individual monosaccharide yields were monitored with gas chromatography and colorimetry. Lignin was determined as the acid insoluble residue. Cell wall composition on a dry weight basis was 17.0% lignin, 32.75% glucose, 28.77% xylose, 7.21% arabinose, 1.11% galactose and 0.35% mannose. Linear xylans and glucans represent a large portion of the cell wall. The quantity of lignified tissue is extraordinarily large, resulting in a rigid, impermeable seed case.

Bracteole cell walls were delignified with acid chlorite. A comparison of enzyme catalyzed hydrolysis of the untreated cell walls with delignified cell walls followed. Three commercial enzyme preparations were incubated with the samples separately and in combination treatments: cellulase (EC 3.2.1.4) from Trichoderma viride supplemented with B-D-glucosidase (EC 3.2.1.21); crude-hemicellulase from Aspergillus niger; polygalacturonase

(EC 3.2.1.15) from Aspergillus niger. The production of reducing groups was monitored. Enzyme catalyzed hydrolysis of lignified cell walls was limited: cellulase followed by polygalacturonase degraded 14.03% of the lignified cell wall, all other treatments yielded less than 10% degradation. Dramatic improvements in the activity of all enzymes were noted after delignification. Cellulase activity increased to 35%, hemicellulase to 25%, and polygalacturonase to 36% hydrolysis of the sample.

Intact seeds were subjected to delignification with acid chlorite at 35 degrees. Of these seeds, half were treated with cellulase, supplemented as before. The germination percentages of delignified and delignified/enzyme treated seeds were compared with other common germination pre-treatments. Four hundred seeds of each treatment were sown in soil and allowed to germinate with 20 degree days and 15 degree nights. The thirty minute delignification left the seed viable, but still lignified: only 3% germinated as compared with 28.0% for physically scarified seeds, 6% for acid treated seeds, 5.5% for surface sterilized seeds, and 4.0% for cold water leached seeds. Physical scarification proved to be the easiest method of pre-treatment.

Cell wall hydrolysis

The cell wall study was designed primarily to indicate the relative ratios of polymer types within the bracteoles. The method of hydrolysis was limited by incomplete solubility of cellulose and lignin-carbohydrate complexes. At the same time, it was confounded

by the overlapping solubilities of hemicellulose and cellulose. Lignin determinations were probably overestimated since nitrogen was not extracted from the plant sample prior to the two-step acid hydrolysis.

An outline of a more detailed cell wall composition study is as follows:

1) delignification with thioglycolic acid and analysis using UV spectral qualities and staining characteristics (Sarkanen and Ludwig, 1971). These methods would allow determination of the lignin component free from interference by other non-lignin polyphenols and protein. In addition, the color reactions will be indicative of the phenylpropane units within the molecule (Wardrop, 1971).

2) isolation and separation of the hemicellulose fraction into hemicellulose A, B, and C components (Blake et al. 1971) followed by acid hydrolysis of the fractions (Bittner et al. 1980) and methylation analysis (Lindberg, 1972), and

3) extraction of the -cellulose residue with 4-methylmorpholine N-oxide (MMNO) (Josleau et al. 1981) followed by acid hydrolysis (Bittner et al. 1980) and methylation analysis (Lindberg, 1972). Fractionation prior to analysis provides a basis for the correlation of monosaccharides released on hydrolysis and polymeric origin. The contamination of hemicellulosic-glucose with cellulosic-glucose is reduced. Complete recovery of the -cellulose residue from MMNO will allow for a good estimation of cellulosic-glucose. Any contamination by the pectic fraction would be easily identifiable as non-glucose peaks after methylation analysis. The use of

methylation analysis provides quantitative information about the isolated polymers' component structure. This information can be compared to methylation analysis after chemical modification of the sample (ie. enzymolysis) to yield information on the linkage characteristics of the polymer.

Biological degradation of the intact seed

Delignification tolerance of the seed is questionable. A study of embryo viability using tetrazolium staining followed by germination trials after varying periods of time in acidic chlorite at 35 and/or 25 C should indicate the utility of pursuing this avenue of research. Assuming delignification is tolerated by the embryo, the effect of enzyme treatment should be assessed with a modification of incubation conditions. Complete immersion of Atriplex seed in enzyme solution for the 5 day period of incubation proved unsatisfactory for two reasons: prolonged exposure to anaerobic conditions and higher than normal temperatures (37 C).

The following modification of the incubation method would enhance the likelihood of success: using glass wool as a substrate, add a highly concentrated solution of cellulase to the imbibition water. The seed should not be submerged, nor should a film of water form around the seed. Preferably, the cellulase solution should be added in small amounts, over a period of time. After 24, 48, and 72 hours, the seed should be transferred to soil for germination. (Seedlings do not transfer well.) Several different enzymes could be employed, specifically, xylanases and B-1,3-glucanases.

Physical degradation of the intact seed

The fact that the bracteoles physically restrain germination rather than chemically contribute to inhibition (through NaCl) was supported by this study. Although physical scarification was reported to be the most "successful" treatment, the 25% germination of filled seed is still far from adequate for mass production of Atriplex gardneri. The possibility of damage to embryos during hammermill treatment can not be overlooked. The hammermill used in this study was not designed specifically for Atriplex. Preferable to the grinding action of the hammermill, it is suggested that the seed be sandwiched between two layers of sandpaper that vibrate in a circular motion with a minimum of pressure on the seed. Failing that, the involvement of genetic factors in the inherent quality of the seed, length of storage time as it affects viability, and hormonal control of germination are possible considerations for further study.

LITERATURE CITED

Adams, G.A. 1965. Lignin determination. Meth. Carbohyd. Chem. 5:185-187.

Albersheim, P. 1976. The primary cell wall, pp. 225-274. In: J. Bonner and J. Varner (Eds.) Plant Biochemistry. Academic Press, N.Y.

Aspinall, G.O. 1980. Chemistry of cell wall polysaccharides, chap. 12. In: J. Preiss (Ed.) The Biochemistry of Plants, vol. 3. Academic Press, N.Y.

Aspinall, G.O., T.N. Krishnamurthy, and K.G. Rosell. 1977. A fucogalactoxyloglucan from Rapseed hulls. Carbohyd. Res. 55:11 (as cited by Aspinall, 1980).

Association of Official Seed Analysts. 1970. Tetrazolium testing handbook for agricultural seeds. Proceedings of Official Seed Analysts. 60(29). 62 p.

Atalla, R.H. 1979. Conformational effects in the hydrolysis of cellulose. Adv. Chem. 181:55-70.

Azuma, J., N. Takahashi, and T. Koshijima. 1981. Isolation and characterization of lignin-carbohydrate complexes from the milled wood lignin fraction of Pinus densiflora SIEB ET ZUCC. Carbohyd. Res. 93:91-104.

Barras, D.R., A.E. Moore, and B.A. Stone. 1969. Enzyme-substrate relationship among B-glucan hydroases. Adv. Chem. 95:119-123.

Bauer, W.D., K.W. Talmadge, K. Keegstra, and P. Albersheim. 1973. The structure of plant cell walls. II. The hemicellulose of the walls of suspension-cultured Sycamore cells. Plant Phys. 51: 174-187.

Beadle, N.C.W. 1952. Studies in halophytes. I. The germination of the seedlings of five species of Atriplex in Australia. Ecology 33:49-62.

Bewley, J.D. and M. Black. 1978. Physiology and biochemistry of seeds: in relation to germination, vol. 1. Springer Verlag, N.Y.

Bisaria, V.S. and T.K. Ghose. 1981. Biodegradation of cellulosic materials: substrates, microorganisms, enzymes, products. Enzyme Microb. Technol. 3:90-104.

Bittner, A.S. 1980. The development of methodology for gas liquid chromatographic determination of plant sugars in maturing Reed Canarygrass. Logan, Utah, Utah State Univ. Dissertation. 151p.

Bittner, A.S., L.E. Harris, and W.F. Campbell. 1980. Rapid N-methylimidazole-catalyzed acetylation of plant cell wall sugars. J. Agric. Food Chem. 28:1242-1245.

Blake, J.D., P.T. Murphy, and G.N. Richards. 1971. Isolation and A/B classification of hemicelluloses. Carbohyd. Res. 16:49-57.

Blumenkrantz, N. and G. Asboe-Hansen. 1973. New method for quantitative determination of uronic acids. Anal. Biochem. 54:484-489.

Brice, R.E. and I.M. Morrison. 1982 The degradation of isolated hemicelluloses and lignin-hemicellulose complexes by cell-free rumen hemicellulases. Carbohyd. Res. 101(1):93-100.

Buchala, A.J. and G. Franz. 1974. A hemicellulosic B-glucan from the hypocotyls of Phaseolus aureus. Phytochem. 13:1887-9.

Caraway, W.T. 1976. Carbohydrates, chap. 6. In: N. Tietz (Ed.) Fundamentals of Clinical Chemistry. W.B.Saunders Co, Philadelphia.

Chang, Martin. 1971. Folding chain model and annealing of cellulose. J. Polymer Sci. 36C:343-362.

Chatterton, N.J.,and C.M. McKell. 1969. Atriplex polycarpa: germination and growth as affected by sodium chloride in water cultures. Agron. J. 61:448-450.

Chatterton, N.J., J.R. Goodwin, C.M. McKell, R.V. Parker, and J.M. Rible. 1971. Monthly variation in the chemical composition of Desert Saltbush. J. Range Mgt. 24:37-40.

Chesson, A. 1981. Effects of sodium hydroxide on cereal straws in relation to the enhanced degradation of structural polysaccharides by rumen microorganisms. J. Sci. Food Agric. 32 (8): 745-758.

Clarke, A.E., R.L. Anderson, and B. Stone. 1979. Form and function of arabinogalactans and arabinogalactan-proteins. Phytochem. 18:521-540.

Collings, G.F. and M.T. Yokoyama. 1979. Analysis of fiber components in feeds and forages using gas-liquid chromatography. J. Agric. Food Chem. 27:373-377.

Collings, G.F., M.T. Yokoyama, and W.G. Bergen. 1978. Lignin as determined by oxidation with sodium chlorite and comparison with permanganate lignin. J. Dairy Sci. 61:1156-1160.

Cornelius, D.R. and L.O. Hylton. 1969. Influence of temperature and leachate on germination of Atriplex polycarpa. Agron. J. 61:209-211.

Cote, W.A. 1977. Wood ultrastructure. Phytochem. 11:1-44.

Cowling, E.B. and W. Brown. 1969. Structural features of cellulosic materials in relation to enzymatic hydrolysis. Adv. Chem. 95:152-187.

Crawford, D.S. and R.L. Crawford. 1980. Microbial degradation of lignin. Enzyme Microb. Technol. 2:11-23.

Crofts, K.A. 1977. The importance of utricle-related factors in germination and seedling vigor of four species of perennial Atriplex. Logan, Utah, Utah State Univ. Thesis. 99p.

Das, N.N., S.C. Das, A.S. Dutt, and A. Roy. 1981. Lignin-xylan ester linkage in Jute fiber (Corchorus capsularis). Carbohyd. Res. 94:73-82.

Davis, G.L. 1966. Systematic Embryology of the Angiosperms, pp. 81-2. John Wiley & Sons, N.Y.

Dewey, D.Y.R. and M. Mandels. 1980. Cellulases: biosynthesis and applications. Enzyme Microb. Technol. 2:91-102.

Dietrichs, H.H. and K.I. Zschirnt. 1972. Investigations on the enzymatic degradation of holocelluloses in vitro. Holz Roh Werk 30: 66-74. (written in German).

Doesburg, J.J. 1973. The pectic substances. Phytochem. 1:270-296.

Dorn, R.D. 1977. Manual of the Vascular Plants of Wyoming, v. 1, p 222. Garland Publ Co., N.Y.

Esau, K. 1953. The Anatomy of Seed Plants, Second Edition, pp. 617-23. John Wiley & Sons, N.Y.

Fleming, I.D. and H.F. Pegler. 1963. The determination of glucose in the presence of maltose and isomaltose by a stable, specific enzymic reagent. Analyst 88:967-968.

Ford, C.W. 1978. Effect of partial delignification on the in vitro digestibility of cell wall polysaccharides in Digitaria decumbens (Pangola Grass). Aust. J. Agric. Res. 29:1157-1166.

Frederick, M.M., J.R. Frederick, A.R. Fratzke, and P.J. Reilly. 1981. Purification and characterization of a xylobiose and xylose-producing endo-xylanase from Aspergillus niger. Carbohyd. Res. 97:87-103.

Freudenberg, K. 1966. Analytical and biochemical gackground of a constitutional scheme of lignin. Adv. Chem. 59:1-21.

Ghose, T.K. and A.N. Pathak. 1973. Cellulases - 2: applications. Process Biochem. 8:20-21.

Greenway, H. 1968. Growth stimulation by high chloride concentrations in halophytes. Israel J. Bot. 17:169-177.

Griffiths, D.W. 1981. The polyphenolic content and enzyme inhibitory activity of testas from Bean (Vicia faba) and Pea (Pisum spp.) varieties. J. Sci. Food Agric. 32(8):797-804.

Gritzali, M. and R.D. Brown, Jr. 1979. The cellulase system of Trichoderma. Adv. Chem. 181:237-260.

Gross, G.G. 1980. The biochemistry of lignification. Adv. Bot. Res. 8:26-63.

Hall, P.L. 1980. Enzymatic transformation of lignin: 2. Enzyme Microb. Technol. 2:170-176.

Hartley, R.D. 1978. The lignin fraction of plant cell walls. Am. J. Clin. Nutr. 31(10): S90-S93.

Hartley, R.D. and M.S. Dhanoa. 1981. Rates of degradation of plant cell walls measured with a commercial cellulase preparation. J. Sci. Food Agri. 32(9):849-856.

Heydecker, W. 1977. Stress and seed germination: an agronomic view, pp. 237-276. In: A.A. Khan (Ed.) The Physiology and Biochemistry of Seed Dormancy and Germination. North-Holland Publ. Co., N.Y.

Holmgren, A.H. and J.L. Reveal. 1966. Checklist of the Vascular Plants of the Intermountain Region. USDA For. Ser. Res. Paper INT-32. 160 p.

Humphrey, A.E. 1979. The hydrolysis of cellulosic materials to useful products. Adv. Chem. 181:25-54.

Jensen, W.A. 1962. Botanical Histochemistry, chap 9. W.H. Freeman & Co., San Francisco.

Joseleau, J., G. Chambat, and B. Chumpitazi-Hermoza. 1981. Solubilization of cellulose and other plant structural polysaccharides in 4-methylmorpholine N-oxide: an improved method for the study of cell wall constituents. Carbohyd. Res. 90:339-344.

Karr, A.L., Jr., and P. Albersheim. 1970. Polysaccharide-degrading enzymes are unable to attack plant cell walls without prior action by a "wall-modifying enzyme." Plant Phys. 46:69-80.

Keegstra, K., K.W. Talmadge, W.D. Bauer, and P. Albersheim. 1973. The structure of plant cell walls. III. A model of the walls of suspension-cultured Sycamore cells based on the interconnections of the macromolecular components. Plant Phys. 51:188-196.

Kilburn, D.M. and P.M. Taylor. 1969. Effect of sulfhydryl reagents on glucose determination by the glucose oxidase method. Anal. Biochem. 27:555-558.

Kirk, T.K. and H.M. Chang. 1981. Potential applications of bioligninolytic systems. Enzyme Microb. Technol. 3:189-196.

Koller, D. 1957. Germination-regulating mechanisms in some desert seeds. IV. Atriplex dimorphostegia (Kar et Kir) Ecology 38:1-13.

Kooiman, P. 1961. The constitution of Tamarindus-amyloid. Recueil Trav. Chim. Pay-Bas 80:849. (as cited by Aspinall, 1980).

Lai, Y.Z. and K.V. Sarkanen. 1971. Isolation and structural studies, chap. 5. In: K.V. Sarkanen and C.H. Ludwig (Eds.) Lignins, Occurrence, Formation, Structure, and Reactions. Wiley Interscience, N.Y.

Lailhacer-Kind, S. and H.M. Laude. 1975. Improvement of seed germination in Atriplex repanda (Phil). J. Range Mgt. 28:491-494.

Lawrence, G.H.M. 1951. Taxonomy of Vascular Plants. MacMillan Co., N.Y.

Li, L.H., R.M. Flora, and K.W. King. 1965. Individual roles of cellulase components derived from Trichoderma viride. Arch. Biochem. Biophys. 111:439-447.

Lindberg, B. 1972. Methylation analysis of polysaccharides. Methods Enzymol. 28:178-194.

MacRae, J.C. 1971. Quantitative measurement of starch in very small amounts of leaf tissue. Planta 96:101-108.

McNeil, M., A.G. Darvill, and P. Albersheim. 1979. The structural polymers of the primary cell walls of dicots. Fortschritte Chem. Organ. Natur. 37:191-249.

Mill, M.A. and R. Tuttobello. 1961. The pectic enzymes of Aspergillus niger 2. Endopolygalacturonase. Biochem. J. 79:57-64.

Millett, M.S., M.J. Effland, and D.F. Caulfield. 1979. Influence of fine grinding on the hydrolysis of cellulosic materials - acid vs enzymatic. Adv. Chem. 181:71-90.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Bio. Chem. 153:375-380.

Neter, J. and W. Wasserman. 1974. Applied Linear Statistical Models. Richard D. Irwin, Inc., Homewood, Ill.

Nomenclature Committee of the International Union of Biochemistry on the Nomenclature and Classification of Enzymes. 1979. Enzyme Nomenclature 1978. Academic Press, N.Y.

Nord, E.C. and J.E. Whitacre. 1957. Germination of fourwing saltbush seed improved by scarification and grading. USDA Calif. For. Range Exp. Sta. For. Res. Note 125. 5 p.

Northcote, D.H. 1972. Chemistry of the plant cell wall. Ann. Rev. Plant Physiol. 23:113-132.

O'Neil, F.W. 1962. Bleaching, chap. 13. In: E. Libby (Ed.) Pulp and Paper Science Technology. McGraw Hill, N.Y.

Pegg, G.F. 1976. Glucanohydrolases of higher plants: a possible defense mechanism against parasitic fungi, pp. 305-306. In: B. Solheim and J. Raa (Eds.) Cell Wall Biochemistry Related to Specificity in Host-Plant Pathogen Interactions. Columbia Univ. Press, N.Y.

Perry, D.A. and J.G. Harrison. 1974. Studies on the sensitivity of monogerm Sugar Beet germination to water. Ann. Applied Biol. 77:51-60.

Philipp, B., V. Jacopian, and F. Loth. 1979. Influence of cellulose physical structure on the thermohydrolytic, hydrolytic, and enzymatic degradation of cellulose. Adv. Chem. 181:127-144.

Pool, R.J. 1929. Flowers and Flowering Plants, pp. 209-210. McGraw Hill Book Co., N.Y.

Preston, R.D. 1974. Physical Biology of Plant Cell Walls. chap 3. Chapman & Hall, London.

Preston, R.D. 1979. Polysaccharide conformation and cell wall function. Ann. Rev. Plant Physiol. 30:55-78.

Reed, G. 1975. Enzymes in Food Processing, Second Edition, chap 6. Academic Press, N.Y.

Richardson, S.G. and C.M. McKell. 1980. Water relations of Atriplex canescens as affected by the salinity and moisture percentage of processed oil shale. Agron. J. 72(6):946-950.

Rombouts, F.M. and W. Pilnik. 1973. Research on pectin depolymerases in sixties - A literature review. Crit. Reviews Food Technol. 3:1-26.

Rosenburg, S.L. 1980. Fermentation of pentose sugars to ethanol and other neutral products by microorganisms. Enzyme Microb. Technol. 2:185-193.

Salisbury, F.B. and C.W. Ross. 1978. Plant Physiology, Second Edition, pp. 103-4, 220-224. Wadsworth Publ. Co., Belmont, Calif.

Sarkanen, K.V. and C.H. Ludwig. 1971. Definition and nomenclature, chap. 1. In: K. Sarkanen and C. Ludwig (Eds.) Lignins, Occurrence, Formation, Structure, and Reactions. Wiley Interscience, N.Y.

Shallenberger, R.S. and G.G. Birch. 1975. Sugar Chemistry. Avi Publ. Co., Westport, Conn.

Sharma, M.L. 1976. Interaction of water potential and temperature effects on germination of three semi-arid plant species. Agron. J. 68:390-394.

Sinner, M., N. Parameswaran, and H. Dietrichs. 1979. Degradation of delignified Sprucewood by purified mannanase, xylanase and cellulases. Adv. Chem. 181:303-329.

Springfield, H.W. 1966. Germination of Four-Wing Saltbush seeds at different levels of moisture stress. Agron. J. 58:149-150.

Springfield, H.W. 1967. Percentage of filled Four-Wing Saltbush seeds. USDA For. Ser. Res. Note RM-81. 4 p.

Stark, N. and L.D. Love. 1969. Water relations of three warm desert species. Israel J. Bot. 18:175-190.

Talmadge, K.W., K. Keegstra, W.D. Bauer, and P. Albersheim. 1973. The structure of plant cell walls. I. The macromolecular components of the walls of suspension-cultured Sycamore cells with a detailed analysis of the pectic polysaccharides. Plant Physiol. 51:158-173.

Tarkow, H. and W.C. Feist. 1969. A mechanism for improving the digestibility of lignocellulosic materials with dilute alkali and liquid ammonia. Adv. Chem. 95:197-218.

United States Department of Agriculture. 1952. Manual for Testing Agricultural and Vegetable Seeds, Agricultural Handbook 30. U.S. Government Printing Office, Wash., D.C. 440 p.

Van Soest, P.J. 1982. Nutritional Ecology of the Ruminant. O & B Books, Corvallis, Oregon.

Versteeg, C. 1979. Pectinesterases from the orange fruit - Their purification, general characteristics and juice cloud destabilizing properties, pp. 2-3. Agr. Res. Rep. 892. Pudoc, Wageningen, Netherlands.

Vest, E.D. 1952. A preliminary study of some of the germination characteristics of Atriplex confertifolia. Salt Lake City, Utah, University of Utah. Thesis.

Vest, E.D. and W.P. Cottam. 1953. Some germination characteristics of Atriplex confertifolia. Utah Acad. Sci. Arts and Letters Proc. 30:108.

Wardrop, A.B. 1971. Occurrence and formation in plants, chap. 2. In: K. Sarkanen and C. Ludwig (Eds.) Lignins, Occurrence, Formation, Structure and Reactions. Wiley Interscience, N.Y.

Wilder, B.M. and P. Albersheim. 1973. The structure of plant cell walls. IV. A structural comparison of the wall hemicellulose of cell suspension cultures of Sycamore (Acer pseudoplatanus) and of Red Kidney Bean (Phaseolus vulgaris). Plant Physiol. 51:889-893.

Wood, T.M. and S.I. McCrae. 1979. Synergism between enzymes involved in the solubilization of native cellulose. Adv. Chem. 181:181-210.

Woolard, G.R., E.D. Rathbone, and L. Novellie. 1977. DMSO-soluble hemicelluloses from the husk of sorghum grain. Phytochem. 16:961-963.

Young, J.A., B.L. Kay, H. George, and R.A. Evans. 1980. Germination of three species of Atriplex. Agron J. 72:705-709.

APPENDIX

User 3118 Date:26jul82 Time:11:51:13 File: 5

Search Time: 0.416 Prints: 62 Descs.: 37

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Set Items Description
  1  6469 BC=25795
  2  1034 CHENOPOD?
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  4     1 GARDNER()SALTBUSH
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  6 38807 CC=02504
  7133810 CC=51522
  8     0 HEMICELLULOSE()GLUCANS
  9    24 HOLOCELLULOSE
 10    30 XYLOGLUCAN?
 11  1436 AMYLOID?
 12     0 B 1 3 GLUCAN
 13     0 B-1,3 GLUCAN
 14     0 B 1 4 GLUCAN
 15     0 B-1,4()GLUCAN
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 17  1411 GLUCAN?
 18     2 HEMICELLULOSIC()GLUCAN?
 19  1411  17 OR 18
20168821  19 OR 16
 21   811 TANNIN?
 22  1169 LIGNIN?
 23   223 PHENOLIC()ACID?
 24     6 LIGNIN()CARBOHYDRATE()COMPLEX?
 25  2168  21 OR 22 OR 23 OR 24
26169419  25 OR 20
 27  2382  26 AND 5
 28    26 S/MAJ
 29     3  28 AND 26
 30     6  3 OR 4
 31 26294 SEED?
 32     7 SEED()HULL
 33 26294  31 OR 32
 34    15 SEED()HULL?
 35    10  34 AND 26
 36     0  35 AND 5
 37     0  34 AND 5
 38   916 TRICHODERMA?
 39   960 CELLULASE
 40   145 PECTINASE
 41    33 ENDOPOLYGALACTURONASE
 42    44 HEMICELLULASE
 43   130 XYLANASE
 44  1822  38 OR 39 OR 40 OR 41 OR 42 OR 4
 45     0  44 AND 34
 46   677  26 AND 44
 47  1167 ANGIOSPERM?
 48     0  46 AND 47
 49    25  44 AND 5
 50    25  49 NOT 35
 51  4716 CELL()WALL
 52    38  51 AND 5
 53    62  52 OR 50
 54   137  51 AND 44
 55     1  54 AND 5
 56     0 PRISCILLA BURTON
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Print 53/3/1-62

73076689
ULTRASTRUCTURAL AND CYTOCHEMICAL OBSERVATIONS ON CHENOPODIUM-AMARANTICOLOR TRICHOME.
DE VECCHI L; GEROLA F M
ISTITUTO DI SCIENZE BOTANICHE, UNIV. DEGLI STUDI DI MILANO, ITALY.
CARYOLOGIA 34 (1). 1981. 117-127. Codan: CARYA

73065097
FUNGAL DECOMPOSITION OF DESMOSTACHYA-BIPINNATA AND CHENOPODIUM-ALBUM LITTER.
ANEJA K R
DEP. BOT., KURUKSHETRA UNIV., KURUKSHETRA, 132119.
PROC INDIAN NATL SCI ACAD PART B BIOL SCI 47 (1). 1981. 93-95. Codan: PIBSB

73064094
ULTRASTRUCTURE OF LESIONS PRODUCED IN LEAVES OF BETA-VULGARIS BY CERCOSPORIN A TOXIN FROM CERCOSPORA-BETICOLA.
STEINKAMP M P; MARTIN S S; HOEFERT L L; RUPPEL E G
BEET SUGAR DEVELOPMENT FOUNDATION, CALIF.
PHYTOPATHOLOGY 71 (12). 1981. 1272-1281. Codan: PHYTA

73035922
COMPARATIVE CELLULOLYTIC ABILITY OF MICRO FUNGI INHABITING VARIOUS TYPES OF LITTER
ANEJA K R; MEHROTRA R S
DEP. BOTANY, KURUKSHETRA UNIV., KURUKSHETRA.
PROC INDIAN NATL SCI ACAD PART B BIOL SCI 46 (4). 1981. 566-571. Codan: PIBSB

22045655
SECONDARY VACUOLES AND PLASMALEMMA TUBULES IN ROOT AND LEAF CELLS OF SALICORNIA-VIRGINICA CULTURED IN SOLUTIONS OF DIFFERENT SALT CONCENTRATION.
PETERSON T A; HILL R J
PLANT AND SOIL SCI. DEP., UNIV. OF RHODE ISLAND, KINGSTON, R.I. 02881.
21ST ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CELL BIOLOGY, ANAHEIM, CALIF., USA, NOV. 9-13, 1981. J CELL BIOL 91 (2 PART 2). 1981. 408A. Codan: JCLBA

22045653
WATER RELATIONS IN THE EPIDERMAL CELLS OF THE HALOPHYTE SUAEDA-MARITIMA.
TOMOS A D; JONES R G W
ADRAN FIOLEMEG A GWYDDOR PRIDD, COLEG PRIFYSGOL GOGLEDD CYMRU, BANGOR, GWYNEDD LL57 2UW, U.K.
36TH CONFERENCE OF THE GESELLSCHAFT FUER BIOLOGISCHE CHEMIE (SOCIETY FOR BIOLOGICAL CHEMISTRY) ON BIOPHYSICS OF WATER, CAMBRIDGE, ENGLAND, UK, JUNE 29-JULY 3, 1981. HOPPE-SEYLER'S Z PHYSIOL CHEM 362 (9). 1981. 1196. Codan: HSZPA

22033857
INTERACTIONS OF GROWTH PROMOTING RHIZOBACTERIA WITH DELETERIOUS RHIZOSPHERE BACTERIA AND FUNGI
SUSLOW T V; SCHROTH M N
DEP. PLANT PATHOL., UNIV. CALIF., BERKELEY 94720.
72ND ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY AND THE CANADIAN PHYTOPATHOLOGICAL SOCIETY, AUG. 24-28, 1980. PHYTOPATHOLOGY 71 (2). 1981. 259. Codan: PHYTA

72069573
ORGANOGENESIS IN HABITUATED SUGAR BEET BETA-VULGARIS CALLUS AUXIN CONTENT AND PROTECTORS PEROXIDASE PATTERN AND INHIBITORS
KEVERS C; COUMANS M; DE GREEF W; JACOBS M; GASPAR T
LABORATOIRE DE BIOLOGIE VEGETALE, 22, QUAI VAN BENEDEN B-4020 LIEGE, BELGIUM.
Z PFLANZENPHYSIOL 101 (1). 1981. 79-88. Codan: ZSPPA

72062497
QUANTITATIVE ION LOCALIZATION WITHIN SUAEDA-MARITIMA LEAF MESOPHYLL CELLS
HARVEY D M R; HALL J L; FLOWERS T J; KENT B
SCH. OF BIOL. SCI., UNIV. OF SUSSEX, BRIGHTON, SUSSEX BN1 9QG, U.K.
PLANTA (BERL) 151 (6). 1981. 555-560. Codan: PLANA

72051752
FILTRATION CHARACTERISTICS OF BEET COSSETTE MASH FERMENTED BY TRICHODERMA-REESEI
BALTRA I; ACEVEDO F
SCHOOL OF BIOCHEMICAL ENGINEERING, UNIVERSIDAD CATOLICA DE VALPARAISO, VALPARAISO-CHILE.
J FERMENT TECHNOL 59 (1). 1981. 59-64. Codan: JFTED

71062400
FACTORS THAT INFLUENCE THE YIELD STABILITY IN CULTURE AND CELL WALL REGENERATION OF SPINACH SPINACIA-OLERACEA MESOPHYLL PROTOPLASTS
ROSE R J
DEP. BIOL. SCI., UNIV. NEWCASTLE, NEWCASTLE, N.S.W. 2308.,
AUST J PLANT PHYSIOL 7 (6). 1980 (RECD. 1981). 713-726.
Coden: AJPPC

71033712
INDUCTION OF SUPPRESSIVENESS TO RHIZOCTONIA-SOLANI IN SOIL
CHET I; BAKER R
DEP. OF PLANT PATHOL. AND MICROBIOL., HEBREW UNIV. OF JERUSALEM, REHOVOT, ISRAEL.
PHYTOPATHOLOGY 70 (10). 1980. 994-998. Coden: PHYTA

20014619
CHARACTERISTICS OF MUTANT CELLULASE PRODUCING STRAIN OF TRICHODERMA-VIRIDE 44
OSTRIKOVA N A; KONOVALOV S A
ALL-UNION SCI.-RES. BIOENG. INST., MOSCOW, USSR.
APPL BIOCHEM MICROBIOL (ENGL TRANSL PRIKL BIOKHIM MIKROBIOL) 16 (1). 1980. 42-45. Coden: APBMA

70074243
GIBBERELLIN CONTROLLED PECTINIC-ACID AND PROTEIN SECRETION IN GROWING CELLS
FRY S C
DEP. BIOCHEM., UNIV. CAMB., CAMBRIDGE, CB2 1QW, ENGL., UK.
PHYTOCHEMISTRY (OXF) 19 (5). 1980. 735-740. Coden: PYTCA

70074165
PURIFICATION AND SOME PROPERTIES OF CELL WALL BOUND INVERTASES EC-3.2.1.26 FROM SUGAR BEET BETA-VULGARIS SEEDLINGS AND AGED SLICES OF MATURE ROOTS
MASUDA H; SUGAWARA S
DEP. AGRIC. CHEM., OBIHIRO UNIV. AGRIC. VET. MED., OBIHIRO, HOKKAIDO, JPN.
PLANT PHYSIOL (BETHESDA) 66 (1). 1980. 93-96. Coden: PLPHA

70074012
ISOLATION OF PROTOPLASTS AND VACUOLES FROM STORAGE TISSUE OF RED BEET BETA-VULGARIS
SCHMIDT R; POOLE R J
DEP. BIOL., MCGILL UNIV., MONTREAL, QUE. H3A 1B1, CAN.
PLANT PHYSIOL (BETHESDA) 66 (1). 1980. 25-28. Coden: PLPHA

70067383
ISOLATED LEAF PROTOPLASTS AND CALLUS CULTURES OF PLANTS FROM BETA L. PRODUCTION GROWTH DIVISION AND DEVELOPMENT ON FLUID NUTRIENT MEDIA
V"RBANOV M; SLAVOVA J; IVANOVA G; ZAKHARIEV A; ZANTOVA R
RES. PROD. COMPLEX, I. IVANOV INST. SUGAR BEET, SHUMEN, BULG.
FIZIOL RAST (SOFIA) 6 (1). 1980. 25-34. Coden: FIRAD

70057162
CHARACTERISTICS OF THE MUTANT STRAIN OF TRICHODERMA-VIRIDE 44 A CELLULASE PRODUCER
OSTRIKOVA N A; KONOVALOV S A
ALL-UNION BIOTECH. RES. INST., MOSCOW, USSR.
PRIKL BIOKHIM MIKROBIOL 16 (1). 1980. 56-59. Coden: PBMIA

70053671
MESOPHYLL CELL PROPERTIES FOR SOME 3 CARBON AND 4 CARBON PATHWAY SPECIES WITH HIGH PHOTOSYNTHETIC RATES
LONGSTRETH D J; HARTSOCK T L; NOBEL P S
DEP. BOT., LA. STATE UNIV., BATON ROUGE, LA. 70803, USA.
PHYSIOL PLANT 48 (4). 1980. 494-498. Coden: PHPLA

70046536
MECHANISM OF BIOLOGICAL CONTROL IN SOIL SUPPRESSIVE TO RHIZOCTONIA-SOLANI
LIU S-D; BAKER R
DEP. PLANT PROTECT., PINGTUNG INST. AGRIC., PINGTUNG, TAIWAN.
PHYTOPATHOLOGY 70 (5). 1980. 404-412. Coden: PHYTA

70040042
ULTRASTRUCTURE OF THE STIGMA AND STYLE OF SPINACH SPINACIA-OLERACEA CULTIVAR PREVITAL IN RELATION TO POLLEN GERMINATION AND POLLEN TUBE GROWTH/
WILMS H J
VAKGROEP PLANTENCYTOL. MORFOL., LANDBOUWHOGESCH., ARBORETUMLAAN 4, 6703 BD WAGENINGEN, NETH.
ACTA BOT NEERL 29 (1). 1980. 33-48. Coden: ABNRA

70026178
THE VISUALIZATION OF WALL ASSOCIATED GRANULES IN THIN SECTIONS OF HIGHER PLANT CELLS OCCURRENCE DISTRIBUTION MORPHOLOGY AND POSSIBLE ROLE IN CELL WALL BIOGENESIS
OLESEN P
INST. PLANT ANAT. CYTOL., UNIV. COPENH., 83 SOLVGADE, DK-1307, COPENHAGEN, DEN.
Z PFLANZENPHYSIOL 96 (1). 1980. 35-48. Coden: ZSPPA

70019474
ELECTRICAL RESISTANCE AND ION MOVEMENT THROUGH EXCISED DISCS OF SUGAR BEET ROOT BETA-VULGARIS CULTIVAR AMONO TISSUE
AIKMAN D P; HARMER R; RUST T S O
SCH. BIOL. SCI., UNIV. E. ANGLIA, NORWICH NR4 7TJ, NORFOLK, ENGL., UK.
PHYSIOL PLANT 48 (3). 1980. 395-402. Coden: PHPLA

70019030
ELECTRON MICROSCOPY OF THE NEBRASKA ROOT GALLING NEMATODE NACOBBUS-BATATIFORMIS AND SYNCYTIA IN SUGARBEET BETA-VULGARIS ROOTS
SCHUSTER M L; ESTES L W; TOLIN S A
DEP. HORTIC., UNIV. NEBR., LINCOLN, NEBR. 68583, USA.
FITOPATOL BRAS 4 (3). 1979 (RECD. 1980). 401-416. Coden: FIBRD

70019016
ASPECTS OF ANTAGONISM OF TRICHODERMA-VIRIDE TOWARD PYTHIUM-DEBARYANUM
DUMITRAS L; FRATILESCU-SESAN T
INST. CERETARI PENTRU PROT. PLANT., B-DUL ION IONESCU DE BRAD 8, BUCURESTI, ROM.
STUD CERCET BIOL 31 (1). 1979 (RECD. 1980). 63-68. Coden: SCBID

70012254
EFFECT OF NUCLEIC-ACIDS ON THE SOLUBILITY OF CELL WALL BOUND SACCHARASE OF SUGAR BEET BETA-VULGARIS SEEDLINGS
MASUDA H; SUGAWARA S
DEP. AGRIC. CHEM., OBIHIRO UNIV. AGRIC. VET. MED., OBIHIRO, HOKKAIDO, JPN.
AGRIC BIOL CHEM 44 (1). 1980. 69-72. Coden: ABCHA

69080991
HYPER SENSITIVE REACTIVITY OF VARIOUS HOST AND NONHOST PLANT LEAVES TO CELL WALL COMPONENTS AND SOLUBLE GLUCAN ISOLATED FROM PHYTOPHTHORA-INFESTANS
DOKE N; SAKAI S; TOMIYAMA K
PLANT PATHOL. LAB., FAC. AGRIC., NAGOYA UNIV., NAGOYA, AICHI, JPN.
ANN PHYTOPATHOL SOC JPN 45 (3). 1979 (RECD. 1980). 386-393. Coden: NSBGA

69068387
RELATION OF PYTHIUM-OLIGANDRUM TO BACTERIA ACTINOMYCES AND SEVERAL FUNGI INHABITING THE RHIZOSPHERE OF THE EMERGING SUGAR BEET
VESELY D
INST. PLANT PROT., RES. INST. PLANT PROD., DRNOVSKA 507, 16106 PRAHA 6-RUZYNE, CZECH.
ZENTRALBL BAKTERIOL PARASITENKD INFEKTIONSKR HYG ZWEITE NATURWISS ABT MIKROBIOL LANDWIRTSCH TECHNOL UMWELTSCHUTZES 133 (4). 1978. 350-356. Coden: ZBPUD

69040075
PHENOLIC COMPONENTS OF THE PRIMARY CELL WALL AND THEIR POSSIBLE ROLE IN THE HORMONAL REGULATION OF GROWTH
FRY S C
BOT. LAB., UNIV. LEICESTER, LEICESTER LE1 7RH, ENGL., UK.
PLANTA (BERL) , 146 (3). 1979. 343-352. Coden: PLANA

69012186
ULTRASTRUCTURE OF LESIONS PRODUCED BY CERCOSPORA-BETICOLA IN LEAVES OF BETA-VULGARIS
STEINKAMP M P; MARTIN S S; HOEFERT L L; RUPPEL E G
BSDF, CROPS RES. LAB., COLO. STATE UNIV., FT. COLLINS, COLO. 80523, USA.
PHYSIOL PLANT PATHOL 15 (1). 1979. 13-26. Coden: PPPYB

69006276
DECOMPOSITION OF DIPLACINE-FUSCA AND SUAEDA-FRUTICOSA IN SALT AFFECTED SOILS
MALIK K A; FAROOQ-E-AZAM
PAK J BOT 10 (1). 1978 (RECD. 1979). 89-94. Coden: PJBOB

19039475
INHIBITION OF THE SUGAR BEET RHIZOSPHERE MYCO FLORA BY THE MYCO PARASITE PYTHIUM-OLIGANDRUM
VESELY D
VYZKUMNY USTAV ROSTLINNE VYROBY, RUZYNE 161 06 PRAHA 6, CZECH.
OCHR ROSTL 15 (2). 1979. 157-159. Coden: OCROA

19003297
MICROBIOLOGICAL PRESERVATION OF LEGUMINOUS GREEN MASS
ILYALETDINOV A N; AKIMEDIEV G Z
INST. MICROBIOL. VIROL., ACAD. SCI. KAZ. SSR, ALMA-ATA, USSR.
IZV AKAD NAUK SSSR SER BIOL 0 (3). 1979. 427-434. Coden: IANBA

68071220
ESTIMATION OF TOTAL DIGESTIBILITY OF DRIED MAIZE FORAGE DRIED SUGAR BEET PULP DRIED LUCERNE WHEAT BRAN WHEAT STRAW AND GRAPE PULP IN PONIES
WOLTER R; DURIX A; LETOURNEAU J C; CARCELEN M
LAB. NUTR. ALIMENT., EC. NATL. VET. LYON, MARCY L'ETOILE, 69260 CHARBONNIERES, FR.
ANN ZOOTECH (PARIS) 28 (1). 1979. 93-100. Coden: AZOOA

68070081
DETERMINATION OF THE SODIUM POTASSIUM AND CHLORIDE ION CONCENTRATIONS IN THE CHLOROPLASTS OF THE HALOPHYTE SUAEDA-MARITIMA BY NONAQUEOUS CELL FRACTIONATION
HARVEY D M R; FLOWERS T J
SCH. BIOL., UNIV. SUSSEX, FALMER, BRIGHTON, SUSSEX BN1 9QG, ENGL., UK.
PROTOPLASMA 97 (4). 1978 (RECD. 1979). 337-350. Coden: PROTA

68070048
EFFECTS OF GLYOXYLATE GLYCINE AND SERINE ON THE ASSIMILATION OF CARBON-14 DI OXIDE IN MESOPHYLL CELLS OF CHENOPODIUM-ALBUM
BAUMANN G; GUENTHER G
SEKT. CHEM./BIOL., PAEDAGOGISCHE HOCHSCH. KARL LIEBNECHT, WISS. BOT., MAULBEERALLEE 2, DDR-15 POTSDAM, E. GER.
BIOCHEM PHYSIOL PFLANZ (BPP) 174 (2). 1979. 160-168. Coden: BPPFA

68044132
SALINITY EFFECTS ON LEAF ANATOMY CONSEQUENCES FOR PHOTOSYNTHESIS
LONGSTRETH D J; NOBEL P S
DEP. BIOL., UNIV. CALIF., LOS ANGELES, CALIF. 90024, USA.
PLANT PHYSIOL (BETHESDA) 63 (4). 1979. 700-703. Coden: PLPHA

67058297
CELLULASE INSOLUBLE FIBER AS A MEASURE OF UNAVAILABLE ORGANIC MATTER IN CATTLE COMPOUNDS CONTAINING ALKALI TREATED STRAW
ISRAELSEN M; REXEN B; THOMSEN K V
BIOTEK. INST., HOLBERGSVEJ 10, DK-6000 KOLDING, DEN.
ANIM FEED SCI TECHNOL 3 (3). 1978. 227-234. Coden: AFSTD

67058282
EVALUATION OF SEVERAL CROPS AS SOURCES OF LEAF MEAL COMPOSITION EFFECT OF DRYING PROCEDURE AND RAT GROWTH RESPONSE
CHEEKE P R; CARLSSON R
DEP. ANIM. SCI., OREG. STATE UNIV., CORVALLIS, OREG. 97331, USA.
NUTR REP INT 18 (4). 1978. 465-474. Coden: NURIB

67037444
THE COMPLEX FORMATION OF BOUND SACCHARASE OF SUGAR BEET SEEDLINGS WITH CELL WALL STRUCTURAL POLY SACCHARIDES
MASUDA H; SUGAWARA S
DEP. AGRIC. CHEM., OBIHIRO UNIV. AGRIC. VET. MED., OBIHIRO, HOKKAIDO, JPN.
AGRIC BIOL CHEM 42 (8). 1978 1485-1490. Coden: ABCHA

67037443
ADSORPTION OF CYTOPLASMIC AND WALL BOUND SACCHARASE OF SUGAR BEET SEEDLINGS TO CELL WALL
MASUDA H; SUGAWARA S
DEP. AGRIC. CHEM., OBIHIRO UNIV. AGRIC. VET. MED., OBIHIRO, HOKKAIDO, JPN.
AGRIC BIOL CHEM 42 (8). 1978 1479-1484. Coden: ABCHA

67030812
ABSCISSION IN THE TUMBLEWEED KOCHIA-INDICA ETHYLENE CELLULASE AND ANATOMICAL STRUCTURE/
ZERONT M; HOLLANDER E; ARZEE T
GEORGE S. WISE CENT. LIFE SCI., TEL-AVIV UNIV., TEL AVIV, ISR.
BOT GAZ 139 (3). 1978 299-305. Coden: BOGAA

67030778
PURIFICATION OF ENDO POLY GALACTURONASE FROM GEOTRICHUM-CANDIDUM BY CROSS LINKED PECTIN COLUMN CHROMATOGRAPHY
ALIEVA D; RODIONOVA N A; AIMUKHAMEDOVA G B; SHELUKHINA N P; MARTINOVICH L I
A.N. BAKH INST. BIOCHEM., ACAD. SCI. USSR, MOSCOW, USSR.
PRIKL BIOKHIM MIKROBIOL 14 (2). 1978 232-235. Coden: PBMIA

67058510
HISTOCHEMICAL CHARACTERIZATION OF CELL WALL PEROXIDASE DURING LIGNIFICATION
CATESSON A-M; CZANINSKI Y; MONTIES B
C R HEBD SEANCES ACAD SCI SER D SCI NAT 286 (24). 1978 1787-1790 Coden: CHDDA

67040182
SOLUBILITY OF PARTIALLY PURIFIED SACCHARASE BOUND TO CELL WALL OF SUGAR BEET SEEDLINGS
MASUDA H; SUGAWARA S
AGRIC BIOL CHEM 42 (11). 1978 (RECD 1979) 2151-2153 Coden: ABCHA

67008068
EFFECT OF VEGETABLE CROPS ON ACCUMULATION OF FUNGUS ANTAGONIST TRICHODERMA-LIGNORUM IN SOIL
BUTMISTRU L D
IZV AKAD NAUK MOLD SSR SER BIOL KHIM NAUK (1). 1978 86-87 Coden: IMBKB

66045562
THE EFFECT OF SOME AGRICULTURAL FOOD INDUSTRY BYPRODUCTS ON THE ACTIVITY OF PECTINASES PRODUCED BY ASPERGILLUS-NIGER MUTANTS
ILCZUK Z
ZAKL. MIKROBIOL. STOSOW., INST. MIKROBIOL. BIOCHEM., UNIW. MARII CURIE-SKLODOWSKIEJ, LUBLIN, POL.
ANN UNIV MARIAE CURIE-SKLODOWSKA SECT C BIOL 31 1976 (RECD 1978) 159-166. Coden: AUCBA

66032781
STEM ABSCISSION IN TUMBLEWEEDS OF THE CHENOPODIACEAE KOCHIA
BECKER D A
MO. RIVER BASIN COMM., OMAHA, NEBR., USA.
AM J BOT 65 (4). 1978 375-383. Coden: AJBOA

66029868
PREPARATION OF CELL WALL FRACTIONS BY VARIOUS WAYS AND ACTIVITY OF BOUND SACCHARASE IN SUGAR BEET SEEDLINGS
MASUDA H; SUGAWARA S
DEP. AGRIC. CHEM., OBIHIRO UNIV. AGRIC. VET. MED., OBIHIRO, HOKKAIDO, JPN.
AGRIC BIOL CHEM 42 (2). 1978 473-474. Coden: ABCHA

65067280
PURIFICATION AND PROPERTIES OF 2 POLY GALACTURONASES EC-3.2.1.15 FROM TRICHODERMA-KONINGII
FANELLI C; CACACE M G; CERVONE F
ORTO BOT. INST., UNIV. ROME, ROME, ITALY.
J GEN MICROBIOL 104 (2). 1978 305-310. Coden: JGMIA

65055033
FINE STRUCTURAL AND CYTOCHEMICAL CHANGES OCCURRING IN BEET DISCS IN RESPONSE TO WASHING
HALL J L
SCH. BIOL. SCI., UNIV. SUSSEX, BRIGHTON BN1 9QG, ENGL., UK.
NEW PHYTOL 79 (3). 1977 (RECD 1978) 559-566. Coden: NEPHA

65011396
RUBUS HOST RANGE SYMPTOMATOLOGY AND ULTRASTRUCTURAL EFFECTS OF A BRITISH ISOLATE OF BLACK RASPBERRY NECROSIS VIRUS
JONES A T; ROBERTS I M
SCOTT. HORTIC. RES. INST., INVERGOWRIE, DUNDEE, SCOTL., UK.
ANN APPL BIOL 86 (3). 1977 381-388. Coden: AABIA

15025169
BIOLOGICAL PROTECTION OF SPROUTING SUGAR BEET AGAINST BLACK ROOT
VESELY D
FOLIA MICROBIOL 22 (6). 1977 (RECD 1978) 456 Coden: FOMIA

64065020
EFFECT OF FUSICOCCIN ON PLANT CELL CULTURES AND PROTOPLASTS
ROLLO F; NIELSEN E; SALA F; CELLA R
PLANTA (BERL) 135 (2). 1977 199-202. Coden: PLANA

64054552
THE DIGESTIBILITY AND VOLUNTARY INTAKE OF THE LEAVES OF TREES AND SHRUBS BY SHEEP AND GOATS
WILSON A D
AUST J AGRIC RES 28 (3). 1977 501-508. Coden: AJAEA

64029111
THE ULTRASTRUCTURE OF POLYMYXA-BETAE ZOO SPORE EXIT TUBE DIFFERENTIATION
D'ANDIRA V; MUTTO S
CAN J BOT 55 (7). 1977 831-839. Coden: CJBOA

63067593
ULTRASTRUCTURE OF ASSIMILATING ORGANS OF SOME SPECIES OF THE FAMILY CHENOPODIACEAE PART 2
VOZNESENSKAYA E V
BOT ZH (LENINGR) 61 (11). 1976 (RECD 1977) 1546-1557. Coden: BOTZA

63065118
METABOLISM OF SUCROSE AND SUGAR ACCUMULATION IN THE SUGAR BEET ROOT
PAVLINOVA O A
FIZIOL BIOKHIM KUL'T RAST 8 (5). 1976 451-461. Coden: FBKRA

63064926
PROPERTIES OF A PROTEIN ACTIVATOR OF NAD KINASE FROM PLANTS
MIIO S; MIYACHI S
PLANT PHYSIOL (BETHESDA) 59 (1). 1977 55-60. Coden: PLPHA

63041445
SOME EFFECTS OF HUMIC-ACID ON CATION UPTAKE BY PARENCHYMA TISSUE
VAUGHAN D; MACDONALD I R
SOIL BIOL BIOCHEM 8 (5). 1976 415-421. Coden: SBIOA

63007868
CELLULASE BIOSYNTHESIS BY GEOTRICHUM-CANDIDUM
KUDRYASHOVA T I; FENIKSOVA R V; TIUNOVA N A
PRIKL BIOKHIM MIKROBIOL 12 (3). 1976 339-344. Coden: PBMIA

77015555
ULTRASTRUCTURAL CHANGES IN THE MESOPHYLL OF LEAVES OF BETA-VULGARIS IN CONNECTION WITH ASSIMILATE TRANSPORT
KURSANOV A L; PARAMONOVA N V
SOV PLANT PHYSIOL (ENGL TRANSL FIZIOL RAST) 23 (2). 1976 242-248 Coden: SOPPA